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A TRIPLE-ALLELE SERIES AND PLUMAGE COLOR IN TURKEYS

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FIVE subspecies of the wild turkey (*Meleagris gallopavo*) which have the same general plumage color pattern but differ in details of color are recognized, and a sixth has been proposed by MOORE (1938). Apparently mutations affecting plumage color have not been reported in the wild turkey except in a state of semi-domestication (DARWIN 1900, p. 304). Those that have been reported in the literature on domestic turkeys prior to 1939 have all been known for a long time (for example, see BROWN 1906).

REVIEW OF THE LITERATURE

Domesticated turkeys are usually divided on the basis of plumage color into varieties, six of which are recognized by the American Poultry Association and described in the "Standard of Perfection." These are the White, which may have flecks or spots of color, and five colored varieties comprising the Bronze, Black, Slate, Bourbon Red, and Narragansett. ROBERTSON, in a series of abstracts of which the earliest was in 1922, summarized conclusions arrived at after much work on the inheritance of these plumage colors. The final conclusions were published in the 1929 abstract. The data obtained by ROBERTSON have recently been published by BOHREN and WARREN (ROBERTSON, BOHREN, and WARREN 1943). The latter authors have used the symbol *cc* for white instead of the *ww* of ROBERTSON (1929), and, like MARSDEN and MARTIN (1939), have adopted the symbol *D* for dominant slate.

The genotypes of the six varieties mentioned may be summarized as follows, using the same symbols as ROBERTSON, BOHREN, and WARREN (1943), who have published excellent photographs of the six varieties and of the various combinations obtained when these varieties are crossed: *cc* (white) is an autosomal recessive to colored; the colored varieties are all *CC*. Thus, females of the Bronze variety are typically *CC bb RR dd N-*. The colored varieties mentioned differ from Bronze as follows: Black is *BB*, Slate is *DD*, but here the preferred exhibition type is also *BB* or a blue slate (ROBERTSON, BOHREN, and WARREN 1943; JAAP and MILBY 1944); the Bourbon Red is *rr*; the Narragansett female is *n-*, the male *nn*, this being the only variety that differs from the Bronze by a sex-linked gene. These genotypes may be considered well established.

In addition to the above, the recessive slate gene *sl* (ASMUNDSON 1940) produces effects similar to those of *D*, although *slsl* slates are lighter in color than *DD* or *Dd* slates. The Black-winged bronze has also been shown to differ by a single pair of genes (*ll*) from the Bronze and other varieties with barred wings (ASMUNDSON 1939). Finally, HUTT and MUELLER (1942) have reported sex-linked recessive imperfect albinism (*alal*), a sublethal. This makes a known

total of seven autosomal genes and two sex-linked genes influencing plumage color in turkeys. However, as will be shown in this paper, b^l is an allele of b and B, which thus belong to a triple allele series. It seems better, therefore, to assign the symbol b^l to the black-winged bronze pattern mutation.

It has been demonstrated (see particularly ROBERTSON, BOHREN, and WARREN 1943; ASMUNDSON 1939) that the various genes interact in different ways to produce new combinations, so that the colors now known may be extended by different combinations of the known genes.

EXPERIMENTAL

The data to be presented in this paper fall into the following groups: (I) Data concerning crosses involving the triple-allele series which comprises the Black (bb, Bb, or Bb^l), the Bronze (bb or bb^l), and the Black-winged bronze (b^lb^l). (II) The interaction of the genes of the triple-allele series with (*a*) other previously reported genes and (*B*) previously unreported genes. Classifications were made on the basis of down colors and checked with plumage colors wherever possible. Not all of the data necessary for a complete study of this sort are yet available, but sufficient has been accumulated to extend materially knowledge of inheritance of down and plumage color in this species.

I. THE TRIPLE-ALLELE SERIES

Stock.—The Bronze were of different strains maintained by the Division of Poultry Husbandry of the UNIVERSITY OF CALIFORNIA for experimental work. Three strains have been used, but since there are no important differences in these strains with respect to plumage color, they need not be considered separately. It is of interest to note that one of these, the so-called "broadbreasted" strain of turkey, is claimed to be descended from stock imported from England over two decades ago and to have been kept relatively free from admixture with other strains of Bronze. It is to be doubted, however, that the claim made by some breeders that there has been no admixture with other strains can be accepted. The Black turkeys were obtained from a breeder who had selected these for exhibition purposes. The origin of the Black-winged bronze has been given elsewhere (ASMUNDSON 1939).

The bronze pattern is essentially the wild pattern. The Bronze differ from the Eastern wild turkey, *Meleagris gallopavo silvestris*, in the amount of brown pigment, the color presumably being more like that of the Mexican wild turkey, *Meleagris gallapavo gallopavo*, which has much less brown than *M. g. silvestris*. Thus the Eastern wild turkey has brown tipped saddle feathers while those of the Mexican and the Bronze are typically white tipped. The differences are determined by multiple genes, according to LEOPOLD (1944) who has found the hybrids between the domestic Bronze and the *silvestris* to be intermediate. The Black-winged bronze had not been reported prior to 1939, but it presumably occurred a long time ago (ASMUNDSON 1939). This mutation has also been identified in the strain of broadbreasted Bronze used (see below), but since, as stated above, the exact origin of this strain is not certain, that does not necessarily prove that the mutation traces back to stock imported from England.

It has already been shown by ROBERTSON, BOHREN, and WARREN (1943) that the Bronze differs from the Black by a recessive autosomal gene. Moreover, it has been shown (ASMUNDSON 1939) that the differences of the black-winged bronze pattern from that of the bronze are determined by a single pair of autosomal genes, the latter being dominant.

Table 1 presents the results of crossing the Black-winged bronze and the Black varieties. The 65 F1's were Black. There was no difference between the progenies from reciprocal matings which agreed with expectation, since both the genes for Black and Black-winged bronze were known to be autosomal. In the F2 generation the numbers obtained are in good agreement with expectation on the basis of three Black to one Black-winged bronze. The unexpected

TABLE 1

F_2 progeny from matings of Black-winged bronze ♀ ♀ with Black ♂ (mating 1) and reciprocal (mating 2).

ORIGINAL MATING NO.	BLACK		BLACK-WINGED BRONZE	
	OBTAINED	EXPECTED	OBTAINED	EXPECTED
1	75	73.50	23	24.50
2	33	33.75	12	11.25
Total	108	107.25	35	35.75

feature of this table is that no Bronze were obtained in the F2 generation. Since previous crosses had shown that the Black was a simple dominant to Bronze and that Black-winged bronze was a simple recessive to Bronze, it would have been expected that if these were not alleles the Bronze would have been recovered in the F2 generation. The results therefore suggested that the Black-winged bronze formed a triple-allele series with the Black and the Bronze.

To test this theory further, various crosses were made between these three varieties, and in each case a backcross was made onto one or the other of the recessives used. The data are summarized in table 2. They show that, regardless of the original combinations, only two of the three phenotypes could be recovered from any cross involving the three varieties and justify the conclusion that the genes involved (B, b, b^l) form a triple-allele series.

II. INTERACTION OF THE GENES OF THE TRIPLE-ALLELE SERIES WITH OTHER GENES

The interaction of the triple alleles with (A) previously reported genes will first be considered, and then their interaction with (B) previously unreported genes. Under (A) data are available for crosses involving (1) Red (*rr*), (2) White (*cc*), (3) Slate dominant (*D*) and recessive (*slsl*); and under (*B*) the crosses are with (1) Palm and (2) Brown.

A-1. Interaction of genes of the triple-allele series with Red

The interaction of the genes of the triple-allele series with R, r is known from crosses made between the Bourbon Red and the Bronze (ASMUNDSON 1937;

ROBERTSON, BOHERN, and WARREN 1943), the Black (ROBERTSON, BOHREN, and WARREN 1943), and the Black-winged bronze (ASMUNDSON 1939). The Bourbon Red is *bb rr* and thus differs from the Bronze (*bb RR*) by a single pair of autosomal genes. On the Munsell color system the color of the red plumage of the Bourbon Red is a slightly darker "brown" than that of the brown in the tail coverts and tail feathers of the Bronze.

It is described as deep brownish red in the Standard of Perfection of the American Poultry Association. The name Copper was apparently applied at one time to a variety of this color in England (BROWN 1906). The brownish red typical of the plumage of colored birds homozygous for *r* is readily dis-

TABLE 2

Backcrosses involving the Black, Bronze, and Black-winged bronze.

MATING NO.	PARENTS	PROGENY		
		BLACK	BRONZE	BLACK-WINGED BRONZE
1	F_1 (Bronze ♀ × Black ♂) ♀ × Bronze ♂	17	14	
2	F_1 (Bronze ♀ × Black ♂) ♀ × Bronze ♂	7	5	
3	F_1 (Black-winged bronze ♀ × Black ♂) ♀ × Bronze ♂	11	9	
4	F_1 (Black ♀ × Black-winged bronze ♂) ♀ × Black-winged bronze ♂	9		5
5	F_1 (Black ♀ × Bronze ♂) ♀ × Black-winged bronze ♂	32	24	
6	Bronze ♀ ♀ (out of 5) × Black-winged bronze ♂		53	75
7	Black ♀ ♀ (out of 5) × Black-winged bronze ♂	60		55
8	Black-winged bronze ♀ ♀ × Black ♂ (out of 5)	26		21

tinguished from the dark or light brown color found in the tail feathers of birds having the bronze, Black-winged bronze, and Narragansett patterns and throughout the plumage of the Brown turkey. The F1's from Bronze × Bourbon Red are readily distinguished from both parent varieties at all ages. They show much brown in the down, have concentrically penciled, dark brown juvenile plumage and adult plumage similar to that of the Bronze but with lighter flight feathers and brown-tipped saddle and tail feathers. The color of these brown tips is about the same as the brown color in the tail feathers of the Bronze (5 Y.R. 4/8 on the Munsell color system). There is an excellent illustration of the adult plumage in ROBERTSON, BOHREN, and WARREN (1943).

Among the bronze-colored F2 progeny from the cross of Bronze × Bourbon Red previously reported (ASMUNDSON 1937), many had brown-colored tips on the saddle and tail feathers suggestive of the color of *silvestris* but with less brown in the surface color of the feathers. Matings between these birds with brown tips gave progeny most of which showed brown tips, but the color varied and was presumably determined by more than one pair of genes. The results apparently paralleled those found in hybrid turkeys by LEOPOLD

(1944), but since no exact measurements of the variation in color were made, a detailed report of the data is not justified. It is interesting to note, however, that this cross of the Bronze and Bourbon Red brought together or, more plausibly, indicates that the Bourbon Red carries genes similar to those of the Eastern wild turkey in their effect on the color of the tips of the saddle and tail feathers.

The Bourbon Red differs from the Black (*BB RR*) variety in two pairs of autosomal genes (ROBERTSON, BOHREN, and WARREN 1943), the F1's being black-red or rusty-black. ROBERTSON, BOHREN, and WARREN (1943) were able to identify the following genotypes in the F2 generation on the basis of plumage color: black, black-bronze, black-red, bronze, bronze-red, buff, and red. They found that the buffs (*BB rr* or *Bb rr*) had lighter colored plumage, darker eyes

TABLE 3

F2's from Black (BB RR) ♀ × Bourbon Red (bb rr) ♂.

PHENOTYPES	OBTAINED	EXPECTED
Black	84	81
Bronze	31	27
Buff	21	27
Red	8	9

and shanks than the reds. The down color of the buff is also darker than that of the red and has an edging of lighter colored down around the beak typically produced by *B* but not by *bb*, bb^1, or b^1b^1.

Additional data confirming the conclusions of ROBERTSON, BOHREN, and WARREN (1943) are in table 3. Black ♀ ♀ were mated to Bourbon Red ♂ ♂ and 47 black-red F1 progeny obtained. Those raised had markings on the flight feathers suggesting barring, which is typical of birds heterozygous for *B*. The main phenotypes—black (*B R*), bronze (*b R*), buff (*B r*), and red (*b r*)—are reasonably easy to identify in older embryos as well as in poults and growing or mature birds. The homozygous black (*BB RR*) cannot be so easily distinguished from black-bronze (*Bb RR*) and black-red (*BB Rr* or *Bb Rr*) nor the bronze from bronze-red in unhatched embryos. Only the main phenotypes, therefore, are given in table 3, and the numbers in these agree satisfactorily (P = .55) with expectation on the basis of a ratio of 9 black : 3 bronze : 3 buff : 1 red.

Like the Black, the Black-winged bronze (b^1b^1 *RR*) differs from the Bourbon Red in two pairs of autosomal genes (ASMUNDSON 1939). The F1's were bronze-red, while in the F2 generation bronze (and bronze-red), black-winged bronze (and black-winged bronze-red), red, and white-downed red were obtained in a ratio approximating 9 : 3 : 3 : 1.

The white-downed reds (b^1b^1 *rr*)—the new pheno- (and geno-) type—were mated *inter se* and, in accordance with expectation, produced only white-downed red progeny. A white-downed red male was mated to a Black (*BB RR*) female. The 11 progeny obtained were all black-red, as would be expected. An

F1 male was then backcrossed onto white-downed red females, and the four expected combinations—black-red (Bb^l Rr), buff (Bb^l rr), black-winged bronze-red (b^lb^l Rr), and white-downed red (b^lb^l rr)—were obtained in approximately equal numbers, as shown in table 4. The data for this backcross indicate that the *B* and *R* genes are not linked.

The results now available show that *rr* in combination with *B* produces an even dark or chocolate brown down color, while in combination with b^lb^l the down is a pure white. Both have lighter brownish red (buff) plumage than the Bourbon Red but, while the Buff (*B*) birds have predominantly white tail and flight feathers like the Bourbon Red, the feathers on the White-downed red (b^lb^l) are predominantly brownish red but with some black in the flight

TABLE 4

Backcross of F_1 (Black-red—Bb^l Rr—out of Black ♀ × White-downed red ♂) ♂ on White-downed red (b^lb^l rr) ♀.

PHENOTYPES	OBTAINED	EXPECTED
Black-red	15	15.25
Buff	14	15.25
Modified Black-winged bronze	16	15.25
White-downed red	16	15.25

feathers. The latter do not have the darker eyes and shanks of the buff. It is evident from this that, while these two mutant genes both reduce the intensity of brownish red pigmentation in the plumage, they differ significantly in other effects.

A-2. Interaction of the genes of the triple-allele series with White

The pure white down of the white-downed red suggested the desirability of getting further information about the effect of the triple alleles on down colors in white turkeys. A number of matings have been made to get information on this point, particularly since it was observed that, while most white turkeys have a varying amount of buff in the down, some have a pure white down. This question has been discussed briefly in a previous report on the black-winged bronze mutation (ASMUNDSON 1939).

For the present series of crosses, three strains or combinations of strains of white turkeys were used. The first one was derived from a white hen that had segregated out of a commercial flock of Bronze. This hen was otherwise of the same constitution as the Bronze variety. She was mated to Bronze turkeys, and in the F2 generation all of the white progeny obtained had buff down color. Later, White Holland turkeys were obtained from a breeder in whose flock of Bronze the black-winged bronze mutation was observed. Among these were found to be some with pure white down. Finally, the Small White turkeys developed by the U. S. DEPARTMENT OF AGRICULTURE at the National Research Center at Beltsville, Maryland, were obtained.

The results of the crosses made are in tables 5, 6, and 7. Table 5 shows the F2 results from mating a White hen with buff down to a Black male. All of

the 36 F1 progeny were black, while in the F2 generation black, bronze, and white were obtained. The numbers are small but are in fair agreement with expectation (P = .36) calculated on the basis of a ratio of 9 black : 3 bronze : 3

TABLE 5

F_2's from White (buff down) ♀ mated to a Black ♂.

PHENOTYPES	OBTAINED	EXPECTED
Black	16	17.1
Bronze	3	5.7
White (white down)	9	5.7
White (buff down)	2	1.9

TABLE 6

Matings of Black, Bronze, and Black-winged bronze to White with buff or white down.

MATING NO.	PARENTS	PROGENY	
		NO.	PHENOTYPES BASED ON DOWN COLOR
1	White (buff down-bb) ♀ × Bronze (bb) ♂	30	Bronze
2	Bronze (bb) ♀ × White (white down-b^1b^1) ♂	13	Bronze
3	Bronze (bb^1) ♀ × White (white down-b^1b^1) ♂	17	Bronze
		17	Black-winged bronze
4	White (white down-b^1b^1) ♀ × Black-winged bronze (b^1b^1) ♂	8	Black-winged bronze
5	White (buff down-bb) ♀ × Black-winged bronze (b^1b^1) ♂	5	Bronze
6	White (white down-Bb) ♀ × Black winged bronze (b^1b^1) ♂	16	Bronze
		13	Black

TABLE 7

Matings of white-plumaged birds with predominantly buff or with pure white down.

MATING NO.	DOWN COLOR OF PARENTS	PROGENY	
		NO.	DOWN COLOR
1	Buff (bb) ♀ ♀ × White (b^1b^1) ♂	41	Buff
2	White (b^1b^1) ♀ ♀ × White (b^1b^1) ♂	46	White
3	White (b^1b^1) ♀ ♀ × White (b^1b^1) ♂	64	White
4	Buff (bb^1) ♀ ♀ × White (b^1b^1) ♂	16	White
		14	Buff
5	White (Bb) ♀ ♀ × White (Bb) ♂	60	White
		23	Buff
6	Buff (bb) ♀ ♀ × White (Bb) ♂	6	White
		8	Buff

white-downed white : 1 buff-downed white. They are consistent with the interpretation that white is a simple recessive to colored and that the *B* gene changes the buff down of the White to a pure white. This is in agreement with the suggestion of MILBY (1943).

Various matings were made between the Whites and the Bronze and Black-winged bronze varieties. The results of these are given in table 6. The white birds used in matings one to four were a mixture of the original two sources—namely, the white hen that had segregated out of a flock of Bronze and the Whites obtained from a private breeder. The white birds used in matings 5 and 6 were of the Beltsville Small White strain. The results of these matings are consistent with the interpretation that, except for being *cc*, the Whites with buff down have the same constitution as the Bronze—namely, that they are *bb* or bb^l. On the other hand, the white turkeys with white down may be either b^lb^l (black-winged bronze) or *BB*, *Bb*, or Bb^l (black). This is shown, for instance, in mating 3, where a broadbreasted Bronze female was mated to a white-downed White male. The progeny were of either bronze or black-winged bronze color, indicating that the Bronze hen was heterozygous (bb^l). It is also shown by the results of mating 4, where the progeny were all black-winged bronze. The results of mating 5 merely confirm those from other sources showing that the bronze gene *b*, shown by the buff down to be present in the White, is dominant to black-winged bronze (b^l). Since black and bronze progeny were obtained from mating 6, the white female in this case must have been heterozygous (*Bb*).

The matings reported in table 6 demonstrate fairly conclusively that the pure white down may be the result of the presence of either the *B* or the b^l gene. The interaction of these genes in white birds is shown by the data in table 7. In the first four matings, strains one and two were used. The results of mating 1 are consistent with the interpretation that the white down is recessive to buff. Matings 2 and 3 show that when these white-downed birds are mated *inter se*, only white-downed progeny are obtained. In mating 4 the buff-downed parent was heterozygous (bb^l), but, obviously, similar results would be obtained if the white-downed parent were heterozygous for *B*, the dominant gene causing white down.

For matings 5 and 6, Beltsville Small White turkeys were used. In mating 5 white-downed birds out of a mating of white-downed×buff-downed parents were used. The numbers of white- and buff-downed progeny obtained from this mating are in good agreement with those expected on the basis of a ratio of 3 white to 1 buff. Buff down in this case is thus a simple recessive to white down. In mating 6 the White was presumably heterozygous (*Bb*) since both white and buff were obtained.

The results of these various matings then are consistent in showing that in white-plumaged (*cc*) birds the pure white down may be the result of either the dominant (*B*) or the recessive (b^l) gene, whereas the intermediate member of the series, the *b* gene (bronze), produces a buff down.

Blue eyes, observed by MILBY (1943) in his white-downed birds, have been observed in all white-downed birds of the Beltsville strain raised to maturity. Other white-downed birds have not had blue eyes. It may, therefore, be concluded that blue-eyed birds are homozygous or heterozygous for *B*. The color of the eyes of white-downed birds raised to maturity may thus be used to determine whether the white down is caused by the *B* or b^l genes.

A-3. Interaction of the genes of the triple-allele series with Slate

Dominant Slate.—For the crosses involving the dominant slate gene, stock was obtained from the U. S. DEPARTMENT OF AGRICULTURE which was described as Blue. A Bronze female was mated to one of the Blue males. Twenty-three F1 progeny were obtained, of which 11 (5 ♂♂, 5 ♀♀, 1?) were blue and 12 (8 ♂♂, 4 ♀♀) were slate, the former having gray, the latter slatey-brown down. The genotype of the Blue male was, therefore, *Bb DD*. Since separate matings of the Blue and Slate were not made and it was not feasible to segregate the results of the matings, the F2 data are reported in table 8 as either blue or slate and black or bronze. This could be compared with the data published by ROBERTSON, BOHREN, and WARREN (1943), the blue or slate comprising the slate and the black or bronze the non-slate. The results of this mating show a close approximation to the expected segregation on the basis of 3 slate to 1 non-slate and show no evidence of sex linkage.

TABLE 8

Relation of dominant Slate to non-Slate.

PHENOTYPES	OBTAINED				EXPECTED
	♂♂	♀♀	??	TOTAL	
F2 generation (from Bronze ♀ × Blue ♂)					
Blue or Slate	31	31		62	63.75
Black or Bronze	11	12		23	21.25
Backcross: Bronze (*bb dd*) ♀♀ × Blue (*Bb Dd*) ♂					
Blue	27	15	1	43	54.75
Slate	28	22	2	52	54.75
Black	26	38		64	54.75
Bronze	28	29	3	60	54.75

One of the blue males obtained from the cross between the Bronze hen and Blue male was mated to Bronze hens (table 8). The total numbers obtained are a fairly good approximation to the equal numbers of blue, slate, black, and bronze expected (P = .20). The data show no indication of linkage between the genes *B* and *D*, since the new combinations—in this case slate and black—comprise slightly more than half the population obtained.

Recesive Slate.—When recessive slates of the constitution *RR sl sl* were mated to Bourbon Reds (ASMUNDSON 1940), birds with the slate color modified to a reddish slate were obtained in the F2 generation. These apparently resembled what TAIBEL (1933) called lilac. In order to obtain information about the interaction of *B* and *sl*, a Lilac male was mated to Black females. The 44 (14 ♂♂, 16 ♀♀, 14?) F1 progeny were black-red, the same color as the progeny from Black × Red. Data obtained in the F2 generation and from a backcross on Lilac females are in table 9. Some of the birds classified as lilac subsequently proved to have the gene *B* and thus should have been placed with the blue lilac. The difficulty in classifying the slates into the respective

groups may partly account for the poor fit of the observed to the expected numbers (P = .001) in the F2 generation. If the slates are lumped together, the observed numbers do not deviate significantly from expected (P = .07). The deviations from expected among the backcrosses are of borderline significance (P = .03). If the various gene combinations among the backcrosses are considered separately, the old combinations of *B*, *b*, and *R*, *r* (*B R* and *b r*) are equal in numbers to the new combinations (*b R* and *B r*). This is also true

TABLE 9

Matings of recessive Slate to Black and Black-winged bronze.

PHENOTYPES	F_2 FROM BLACK ♀ × LILAC ♂		BACKCROSS LILAC ♀♀ × F_1 ♂
	OBTAINED	EXPECTED	OBTAINED*
Black and/or Black-red (*B R Sl*)	40	48.9	15
Bronze and/or Bronze-red (*b R Sl*)	19	16.3	7
Buff (*B r Sl*)	10	16.3	10
Red (*b r Sl*)	7	5.4	5
Blue (*B R sl*)	17	16.3	6
Slate (*b R sl*)	10	5.4	12
Blue lilac (*B r sl*)	6	5.4	1
Lilac (*b r sl*)	7	1.8	9

F_2's from Black-winged bronze ♀♀ × Slate ♂

PHENOTYPES	OBTAINED	EXPECTED
Bronze	75	72.6
Black-winged bronze	23	24.2
Slate and slate-gray	31	32.3

* Expected 8.1 in each group.

of *R*,*r* and *Sl*,*sl*. In the case of *B*,*b* and *Sl*,*sl*, however, there are 46 of the old combinations (*B Sl* and *b sl*) and only 19 of the new combinations (*b Sl* and *B sl*). If only the non-slates are considered, there were 25 old to 12 new combinations of these genes. Nevertheless, in view of the small numbers, absence of a statistically significant indication of such linkage in the F2 generation, and the uncertainty in the classification of the various slates, this evidence of linkage must be considered tentative until conclusive data are obtained.

From a mating of Black-winged bronze (*b^1b^1 SlSl*) females to a Slate (*bb slsl*) male, 61 (31 ♂♂, 29 ♀♀, 1?) bronze progeny were obtained. The numbers obtained in the F2 generation are in good agreement with the expected numbers on the basis of a 9:3:4 ratio (table 9), and there is no indication of linkage. Here the slates are grouped with the slate-grays (*b^1b^1 slsl*), since they were not readily distinguished on the basis of down color. A female classified as slate-gray on the basis of plumage color—her plumage was a uniform slate-gray throughout—when mated to a Black-winged bronze male gave 12 (6 ♂♂, 5 ♀♀, 1?) black-winged bronze progeny. The data for these various crosses,

though incomplete, indicate that the mutant genes (*B* and b^1) reduce brownish-red in the down and plumage, giving a more uniformly gray plumage.

E-1. Palm

A mutation from the Bronze which has generally been called the Palm variety has been known for several years, and the origin of one strain is described by MARSDEN and MARTIN (1944). Males and females of this color pattern (fig. 1) have been seen fairly frequently in commercial flocks of turkeys

FIGURE 1—The Palm.

in California, indicating that the mutation is widespread and hence occurred a long time ago or has recurred frequently. MARSDEN and MARTIN (1944) suggest (p. 63) that the original mutation occurred in Europe, since birds of this color were exhibited under the name Crollwitz at the Sixth World's Poultry Congress held in Germany in 1936. A pair of Palm birds were obtained from MR. C. G. LEWIS of Victorville, California, in January 1941. These were referred to by MR. LEWIS as Desert Palm. He stated at the time that none had been observed in his flock of Bronze turkeys before.

Black-winged bronze females were mated to the original Palm male. The 27 (11 ♂♂, 12 ♀♀, 4?) F1's were bronze, from which it may be inferred that the palm gene is not an allele in the same series as the triple alleles. The F1's were not mated.

Bronze females were also mated to the original Palm male. The 86 (43 ♂♂, 39 ♀♀) F1's were all bronze, and in the F2 generation the number recovered did not deviate significantly ($P = .05$) from the numbers expected on the basis of 3 bronze to 1 palm. The data (table 10) indicate that the palm gene (p) is a simple autosomal recessive to the bronze or non-palm (P). The original Palm male was also mated to a Black female. All the progeny were black. No F2 progeny were obtained.

Additional data which show the relation of palm (*p*) to non-palm (*P*) were obtained from a mating between Bourbon Red females and a Palm male. The palm behaved in the crosses with the red in much the same way as the bronze or black-winged bronze, the 24 (12 ♂♂, 12 ♀♀) F1's being a modified bronze. The color of the hybrid was somewhat lighter than when the Bronze was crossed with the Bourbon Red, due apparently to relatively less black and more brown. It is evident, however, that the typical Palm is homozygous for the *R* gene, as are the Black, Bronze, Black-winged bronze, and Narragansett varieties. It proved difficult to distinguish red palm (*pp rr*) individuals from modified palm (*pp Rr*) and palm (*pp RR*) on the basis of down color; hence,

TABLE 10

F2 progeny from matings of a Palm male with Bronze and Bourbon Red females.

PHENOTYPES	OBTAINED ♂♂	♀♀	??	TOTAL	EXPECTED
		Bronze ♀♀ × Palm ♂			
Bronze	27	28		55	48
Palm	5	4		9	16
		Bourbon Red ♀♀ × Palm ♂			
Bronze	30	18	3	51	44.5
Palm	5	6		11	14.8
Red	3	5		8	14.8
Red palm	4	5		9	4.9

these were grouped together in the tabulation of the F2 generation. The F2 data (table 10) are in good agreement with expectation on the basis of 1 palm to 3 non-palm, but the agreement with the expected on the basis of 3 bronze:6 bronze-red:4 palm is only fair (P = .04). The plumage of the birds classified as red palm (*pp rr*) was a distinctly pale brownish red in color, the brownish red being distributed about the same as black in the Palm (fig. 1). The gene *p* thus affects the distribution of pigment and also reduces the intensity of brownish red pigmentation.

B-2. Brown

At the same time that the Palm was received, a Brown hen was also obtained from MR. LEWIS. The distribution of the brown pigment in the plumage was the same as that of pigment (black and brown) in the Bronze. This hen was mated to a Bourbon Red male.

The 11 (6 ♂♂, 5 ♀♀) progeny obtained were all modified bronze. The number of progeny obtained in the F2 generation (table 11) was small but consistent with expected numbers (P = .33 for males, .25 for females) and shows that the brown is a sex-linked recessive. The brown color of the Brown hen and her brown descendants is phenotypically distinct from that of the Bourbon Red. The Brown hen when crossed with the Bourbon Red gave progeny resembling those resulting when the Bourbon Red is mated to the Bronze or

other predominantly black varieties. The Brown hen was, therefore, homozygous for *R*, which is incompletely dominant to *r*. On the other hand, the production of the brown pigment typical of the brown mutant, the gene for which may be designated *e*, was apparently completely suppressed by the dominant *E* gene. The results of other matings confirm the conclusion that the *e* gene is a sex-linked recessive.

TABLE 11

F_2 progeny from matings of a Brown female with a Bourbon Red male.

PHENOTYPES	OBTAINED ♂♂	OBTAINED ♀♀	EXPECTED ♂♂	EXPECTED ♀♀
Bronze	6	1	3·75	1·75
Modified bronze	7	7	7·50	3·50
Red	2	1	3·75	1·75
Brown	0	5	0	7·00

DISCUSSION

The new genes reported in this paper increase to nine the number of mutant genes known to influence plumage color. Of these, six (*B*, b^1, *p*, *r*, *D*, and *sl*) are autosomal and three (*n*, *al*, and *e*) are sex-linked. Obviously, the loci thus identified number not more than eight, since two of these genes, *B* and b^1, form a triple-allele series with *b*.

The phenotypes produced by different combinations of the autosomal genes used in the crosses reported in this paper may well serve as a basis for a discussion of their interactions. The information available may be summarized as follows:

(a) *cc*—white plumage
bb, bb^1—buff down
BB, *Bb*, Bb^1, or b^1b^1—white down

(b) *CC* or *Cc*—colored plumage. Some color in down except in b^1b^1rr.

The known plumage patterns produced by autosomal genes are:

	RR	*rr*	*DD*, *Dd*, or *slsl*	
			With *RR* or *Rr*	With *rr*
BB, *Bb*, or Bb^1	Black	Buff	Blue	Grayish lilac
bb or bb^1	Bronze	Bourbon red	Slate	Lilac
b^1b^1	Black-winged bronze	White-downed red	Slate gray	
pp (with *bb*)	Palm	Red-palm		

The most obvious gaps in the tabulation are the combinations produced by *pp* (palm) with *B*, b^1, and with slate (*D* or *slsl*). Some generalizations with

respect to the effects of these genes, however, may be made from the data available.

Any classification of the genes determining plumage and down color in turkeys on the basis of their effect or the way they act must be regarded as, in some degree, arbitrary. Thus they might be classified as pigment genes or pattern genes. The classification of WRIGHT (1942) could also be used by dividing the genetic factors into (1) general factors—those acting on all pigment cells alike, (2) gradient factors, and (3) local factors. As far as plumage color of turkeys is concerned, the *C*, *c* genes are pigment genes that presumably affect all melanophores. It should be noted, however, that pigment production is not completely suppressed in all white (*cc*) birds, since they not uncommonly show black flecks in the plumage. This is presumably due to differences in the viability of the melanophores similar to that found by HAMILTON (1940) in white breeds of the domestic fowl.

The presence or absence of buff pigment in the down color depends on gene interaction. Colored (*CC* or *Cc*) birds have colored down with the exception noted above. Other exceptions may be found when the various combinations of the genes are completed. Some of the birds that later have palm plumage are white with buff similar to that of *cc bb* birds, but they can be distinguished from such whites by black in the outer or primary flight feathers.

It is likely that all the genes determining plumage color in turkeys are to some extent gradient factors in the sense of altering thresholds at which pigment is produced by the melanophores and laid down in the feathers. The genes, other than *C*, *c*, may, however, be conveniently divided into pigment and pattern genes, the former determining primarily the kinds of pigments produced, the latter the amounts and/or distribution of pigments. In the former group are *R*, *r*, and *E*, *e*, the status of *D*, *d* and *Sl*, *sl* being less certain; in the latter group are the autosomal genes, *B*, *b*, b^l and *P*, *p* and the sex-linked genes *N*, *n* and *Al*, *al*.

BOHREN, CONRAD, and WARREN (1943) concluded that blue feather pigment in the domestic fowl is the same as that found in black feathers. Nevertheless, the status of the slate genes in turkeys must be regarded as uncertain despite the statement by JAAP and MILBY (1944) that "The gene, *G*" (*D* in this paper) "in itself appears to have no power to place gray color in the growing feather, but merely changes to gray those areas where black would occur . . . ." Until definite proof is forthcoming, it must remain an open question whether the *D* and the *sl* genes produce a qualitative or a quantitative change in the pigment found in the feathers of slate birds. In favor of the views expressed by JAAP and MILBY are the noticeable differences in plumage color between birds of the constitution *D R* as compared with those that are *D rr*. On the other hand, unpublished spectrophotometric absorption curves plotted for solutions made from the feathers of different varieties of turkeys show differences in the slope of the curves (for example, blue and black). Such differences, according to BOHREN, CONRAD, and WARREN (1943), indicate that the pigments are different. It seems better, however, to defer final judgment until further decisive evidence is available.

One other point should be emphasized regarding slate—namely, that combinations of genes determine the uniformity of slate color (JAAP and MILBY 1944). However, combinations of genes other than those of *B* and *D* mentioned by JAAP and MILBY will produce a uniform gray color—for example, combinations of $b^l b^l$ with *slsl* and *R*. The effects of *B* and $b^l b^l$ on slate plumage are due partly to the reduction or, in some cases, elimination of brown and brownish red pigment from the down and plumage (see below).

The evidence regarding the *R*, *r* pair of genes is fairly clear-cut. These genes primarily determine the production of pigments (*R*—black; *r*—brownish red). The presence of *R* does not appear to inhibit entirely the action of *r*, even in the adult plumage which in *Rr* birds is much more nearly like that of the Bronze than the juvenile plumage. Moreover, birds homozygous for *R* may have much brown in the adult plumage (for example, brown in the tail feathers of Bronze, Black-winged bronze) and also the down and juvenile plumage. In general, the parts of the plumage of Bourbon Reds (*rr*) that are white or partly white correspond to those in Bronze (*RR*) with reduced black (for example, the barred flight feathers) or that are partly brown, as in the tail feathers. The similarities in the distribution of black in the Bronze and brownish red in the Bourbon Red may well be the result of other (pattern) genes which are, so far as known, identical in the two varieties, while the differences may reflect differences in the steps leading to the synthesis of black and brown in the Bronze and of brownish red pigment in the Bourbon Red.

Possible explanations for the production of brown in some sections of the plumage, black in others, have been discussed by WANG (1943, p. 344). In the turkey such combinations of colors are the result of particular gene combinations; these combinations include *R* with *bb*, bb^l or $b^l b^l$ with *N*- or *n*- (*nn* in the male) and with *P* but not *pp*. The gene *B* by extending black to all parts of the plumage precludes the apparent combination of black and of brown pigment in the plumage. Attention has also been called to the fact that the plumage of *rr* $b^l b^l$ birds while predominantly a light brownish-red may show a little black pigment. Males homozygous for *r* (for example, Bourbon Reds) have black tipped breast feathers, while those of the females have white tips. The effect of the sex hormones is the same in *RR* or *Rr* individuals, but only in males of the constitution *rr* does a combination of black with brownish red pigment result.

In contrast to the *R*, *r* pair of genes, the genes of the triple allele series (*B*, *b*, b^l) and the *P*, *p* pair of genes are primarily pattern genes which determine the distribution and perhaps the amount of whatever pigments may be present. They and the pigment genes (*R*, *r*) may be postulated to affect the thresholds above which pigments are produced. The relationships here may be complicated, involving both genetic and nongenetic factors, as brought out by WRIGHT (1942) in his review of this problem in guinea pigs. Sufficient data are not yet available regarding the kinds and amounts of pigments present in the plumage of different genotypes of turkeys. On the basis of the visual evidence it is nevertheless apparent that the distribution of the pigments depends partly on the genes referred to above as pigment genes. Thus in black (*RR*) birds,

those having the *B* gene are most uniformly black throughout, whereas in red (*rr*) birds it is birds of the constitution b^1b^1 that have the pigment spread most uniformly through the plumage.

ROBERTSON, BOHREN, and WARREN (1943) correctly stated with reference to the genes considered by them that the Narragansett gene (*n*) and the black gene (*B*) are the only ones capable of reducing the intensity of red pigment. The new information derived from the matings reported in this paper require a modification of their statement. The Narragansett gene (*n*) appears to be primarily a pattern gene that modifies the intensity and distribution of color, presumably without effecting a qualitative change in the pigments present, and is thus in the group under consideration. It may then be stated that only when the plumage color is determined by the "wild" genes of this group (*b; P; N*) is intense red pigment produced, whereas the mutant genes (*B*, b^1; *p*; *n*) reduce the intensity of red pigmentation, the recessive genes having been observed to do so only when the bird is homozygous for them.

Some other effects of the *R*, *r* and *B*, *b*, b^1 genes may be illustrated by their effects on the apparent presence of pigments in the down of colored (*CC* or *Cc*) turkeys. The following summarizes the data (+ = pigment present; − = pigment absent):

	RR	*Rr*	*rr*	*RR*	*Rr*	*rr*
	Black pigment			Brown or brownish-red pigment		
BB, *Bb*, Bb^1	+	+	−	−	+	++
bb, bb^1	+	+	−	+	+	+
b^1b^1	+	+	−	−	+	−

It is apparent from this somewhat oversimplified summary that, in combination with *RR*, the genes *B* and b^1b^1 reduce the apparent amount of brown pigment in the down. The reduction of brown may also be observed in the plumage of such birds and in the down and plumage of slate (*D* or *slsl*) birds. The tendency to reduce brown pigment may be considered typical of these genes (*B* or b^1b^1) in combination with *RR* and, as noted above, is also observed in the plumage of birds homozygous for *r*. However, the exact effect is the result of gene interaction and may be different in the down from what it is in the plumage. Thus the down color of birds homozygous for *r* with *B* is an even chocolate or dark brown, while with b^1b^1 it is white. The adult plumage of both these genotypes is buff, although, as stated above, they differ in the distribution of the brownish red pigment and in the amount of black visible in the plumage. While the two buff phenotypes are sufficiently alike to be confused on casual inspection, it is likely that so-called buff turkeys have the constitution *BB rr*.

Most turkeys have brown or dark brown eyes. Black (*BB RR*) or buff (*BB rr*) turkeys have darker eyes (presumably more black pigment in the eyes) than bronze (*bb RR*) or brownish red (*bb rr*) birds (ROBERSTON, BOHREN, and WARREN 1943). In white (*cc*) birds *B* changes the eye color to blue, apparently by reducing or eliminating completely the brown pigment. Varying the com-

binations of genes other than *B* causes relatively minor effects such as changing the intensity of brown pigmentation of the eye.

The other two sex-linked genes, *al* (imperfect albinism) and *e* (brown) are apparently pattern and pigment genes, respectively. The exact interaction of these genes with genes other than those typical of the bronze remains to be worked out. The preliminary information from the matings summarized in table 11 indicates, however, that the genes *R* and *r* have little effect on the color of *e* (brown) females. The palm gene (*p*) modifies the distribution of brown pigment, and other data indicate that the *B* gene may modify both the distribution and the intensity of brown pigment.

With respect to linkage relations, it may be considered reasonably well established that the triple-allele series *B*, *b*, b^l is on a different chromosome from *R*, *r*, and *D*, *d* but may be linked with *Sl*, *sl*. Adequate linkage tests have not been made for other autosomal gene combinations, nor for the sex-linked genes.

SUMMARY

The three genes *B* (Black), *b* (Bronze), and b^l (Black-winged bronze) form a triple-allele series, and dominance is in the order given.

White (*cc*) birds of the constitution *BB*, *Bb*, Bb^l, or b^lb^l have white down free from the buff pigment found in *bb* or bb^l whites. Matings of the different strains of white gave ratios of white to buff down that conformed to expectation on the basis of the known relations of the *B*, *b*, b^l genes.

The dominance of the *D* (slate) gene over non-slate (*d*) was confirmed. The *B* and *D* genes are not linked.

Additional evidence was obtained which confirms the finding that *sl* (slate) is a simple recessive to non-slate (*Sl*). *R* and *Sl* are not linked. *B* and *Sl* may be linked, but critical evidence is lacking.

The genes *B* and *R* are not linked.

Palm is a simple autosomal recessive to non-palm. The palm gene (*p*) is at a different locus from that of the triple alleles.

An apparently new mutation, brown, is a sex-linked recessive.

The new genes reported in this paper increase to nine the number of mutant genes known to influence plumage color in turkeys, of which six (*B*, b^l, *p*, *r*, *D*, and *sl*) are autosomal and three (*n*, *al*, and *e*) sex-linked.

The four mutant genes that are primarily pattern genes (*B*, b^l, *p*, and *n*) all reduce the intensity of red pigmentation in the plumage. The mutant genes of the triple allele series (*B* and b^l) produce more uniformly slate birds than the *b* gene.

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FERTILIZATION WITHOUT REDUCTION IN GUAYULE (PARTHENIUM ARGENTATUM GRAY) AND A HYPOTHESIS AS TO THE EVOLUTION OF APOMIXIS AND POLYPLOIDY

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INTRODUCTION

AN UNDERSTANDING of the method of reproduction of the material being bred is essential to any breeding program. Therefore, the presence of plants of *Parthenium argentatum* Gray in which fertilization without reduction in number of chromosomes is occurring must be of decided interest, because of the information concerning the nature and manner of inheritance of the processes of reproduction that can be derived from a study of such plants and their progenies. Also, from such studies it should be possible to obtain some information as to the probable evolution of apomixis and polyploidy, all of which would be of material aid in developing and selecting methods to be used in breeding this crop.

MATERIALS AND METHODS

The material employed in this study was derived from seed collections made in Old Mexico and the Trans-Pecos area of Texas during 1942. Primarily, the studies involve the plants grown from seed collection 42163 and the progenies of these plants. Seed collection 42163 came from an individual plant growing on the 02 Ranch in Texas. In order to obtain some information concerning the natural population of which the plant producing seed collection 42163 is a member, the plants grown from other seed collections of this same population were included in the studies, also. These are the three following collections: a non-selected sample of seed (4278) which included seed from more than 5000 plants, seed collected from 23 plants of the better morphological type, and seed collected from 20 plants of the "intermediate type" (poorer morphological type). The seed from these latter 43 plants was kept and grown as individual plant selections. The seed collection 42163 was taken from one of the 20 "intermediate type" plants. For a description of some of the morphological characteristics and the probable phylogeny of these "intermediate type" plants see ROLLINS (1944). It should be pointed out that most of the plants of this natural population growing on the 02 Ranch had a heavy crop of seed, so that the plants grown from collection 4278 are representative progeny of this natural population.

The experimental design employed in all these studies is a randomized complete block. In the emergence and seedling studies the number of replicates

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was five and the least number of seeds sown per replicate was 100, making a total of 500 seeds per collection or strain. The emergence and seedling studies were conducted in the greenhouse. The plants studied in the greenhouse were grown in an experiment having 29 replicates. Since a number of the weaker plants of some strains died and since some phenotypes were limited in number, in order to get comparable data it was necessary to resort to random sampling of this material. In case of the field grown plants the number of replicates was eight and the number of plants per replicate was 12. The greenhouse plants were started in flats and later transplanted into pots; whereas, the field plants were started in the greenhouse and transplanted from flats into the field.

All operations including the pollen measurements, with the exception of those measured from inside wall of intine to inside wall of intine, were performed on the basis of replicates so as to control any differences that might occur due to procedures, practices, or other environmental agencies. The measurements of diameter of pollen grains from inside wall of intine to inside wall of intine involved plants from a number of experiments and include measurements from both field and greenhouse grown plants. Then, since the plants and collections with different chromosome numbers were randomized within all experiments, these measurements should be a random sample also and hence comparable.

A smear technique developed by DR. JAMES R. MEYER (unpublished) was employed in the cytological studies made by the author. This method was much superior to those in common use.

Microcytes were recorded on the basis of the number occurring among 100 pollen grains of each plant. For further details concerning this character in guayule see POWERS and GARDNER (1945).

Odds of 19 to one or greater against the deviations noted being due to chance were accepted as statistically significant. The "t test" and χ^2 (Chi-square) were employed to test statistical significance of differences between means and percentages, respectively, and Bartletts' test for homogeneity of variances was used to test the significance of differences between variances. As would be necessary, in case of the percentages, the χ^2 values were calculated from the original numbers.

EXPERIMENTAL RESULTS PERTAINING TO THE OCCURRENCE OF ABNORMAL FACULATATIVE AMPHIMIXIS

Types of Plants

Three contrasted types of plants occur in the material studied—namely, normals, aberrants, and hyperaberrants. A further division of the normals into typical normals and off-type normals (morphologically distinguishable from the typical normals and aberrants) materially facilitates the interpretation of the data.

The different types are illustrated photographically in Plate 1. Four normal plants are illustrated in A. The three plants to the left in A are typical

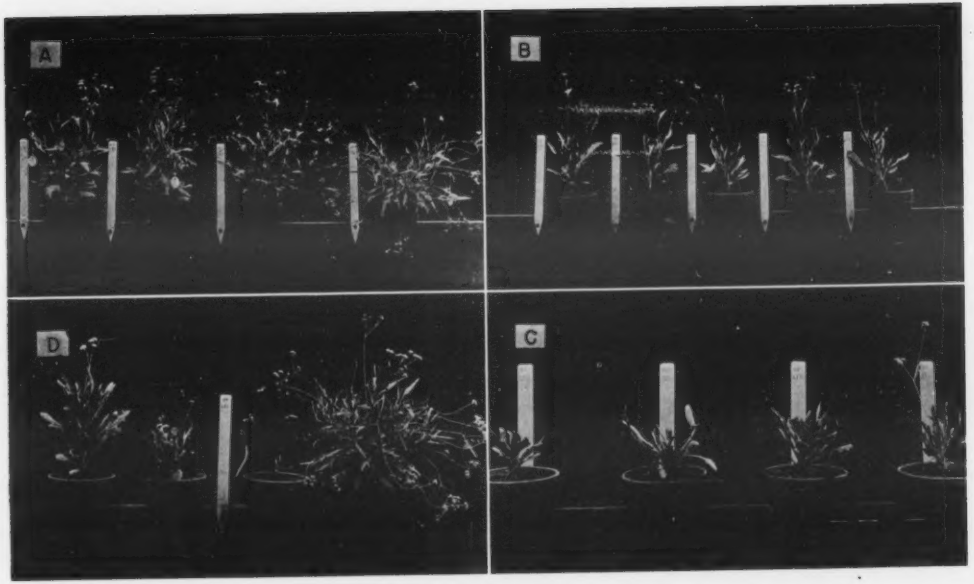

PLATE 1.—Type of Plants: A—normals (typical normals and off-type normals); B—aberrants; C—hyperaberrants; D—aberrant, hyperaberrants, and off-type normal.

normals, and the one to the right is an off-type normal. The aberrant plants are illustrated by B, the hyperaberrants by C, and all three types occurring in one strain by D. In D the plant on the left is an aberrant, the two middle plants are hyperaberrants, and the plant on the right is an off-type normal. The 12-inch pot labels were used in all four photographs to provide perspective for making size comparisons. These plants of any one photograph are comparable as regards each other and with plants of any other photograph because they came from a randomized experiment and are of the same age.

As shown by the photographs, the characteristics of these types are as follows. The normals are the most vigorous, possess uniform growth as to leaf pattern, and flower earlier and more profusely. The aberrants are decidedly less vigorous, possess non-uniform growth as to leaf pattern, are later flowering, and do not flower so profusely. The hyperaberrants, as the name implies, represent an accentuation of the characters of the aberrants. They are less vigorous than the aberrants, show very irregular growth as to leaf pattern, are late flowering, and do not produce many flowering branches. For a further description of the aberrants, the reader is referred to STEBBINS and KODANI (1944) and POWERS and ROLLINS (1945).

Before proceeding further, it is well to have in mind the information relative to the chromosome numbers of these types. The typical normal plants of collection 42163 were found by BERGNER to have 72 chromosomes plus one small chromosome. STEBBINS and KODANI (1944) and POWERS and ROLLINS (1945) have shown that the aberrants arise from the 72±-chromosome plants by the fertilization of non-reduced egg-cells with reduced gametes. Hence, the aberrants would be expected to have 108± chromosomes. BERGNER (unpublished data) found that aberrant plants arising from seed of 72±-chromosome plants may have 90±, 99±, or 108± chromosomes, depending upon the chromosome number of the pollen grain bringing about fertilization. Occasionally aneuploids of the 72±-chromosome group have phenotypes somewhat similar to those of the 90±-, 99±-, and 108±-chromosome aberrants. However, these are of such rare occurrence in the material being reported on in this article as not to merit further consideration.

Frequency of Occurrence of Different Types

The frequency of the occurrence of the different types in the first, second, and third generations of the plant from which seed collection 42163 was made should yield considerable information concerning methods of reproduction occurring in this plant and in progenies resulting from it, and information also concerning the manner of inheritance of some of the processes involving methods of reproduction.

The percentages of normal, aberrant, and hyperaberrant plants in the accessions and strains are given in table 1. The data are for accession 42163, 23 better-type individual plant selections, 19 intermediate-type individual plant selections, accession 4278, accession 42441 (variety 593), and strains which are the progeny of plants derived from seed collection 42163 and which, therefore, are the second generation of the original plant from which seed collection

42163 was taken. The seed which produced the progeny of the plants derived from seed collection 42163 came from two seed crops, that of 1943 and that of 1944.

TABLE 1

The percentages of normal, aberrant, and hyperaberrant plants in accession 42163, in the strains which are the progeny of this accession, in other collections from the 02 ranch in Texas, and variety 593. The data are for 1943 and 1944. No asterisk designates individual plant collections of seed, one asterisk individual plant collections of seed bulked to form a mass selection, and two asterisks a non-selected sample of seed collected at random from the population.

ACCESSION OR STRAIN	1943				1944			
	NORMALS	ABERRANTS	HYPERABERRANTS	PLANTS CLASSIFIED	NORMALS	ABERRANTS	HYPERABERRANTS	PLANTS CLASSIFIED
	Percentage	Percentage	Percentage	Number	Percentage	Percentage	Percentage	Number
Collections from 02 ranch in Texas and variety 593								
A 42163	9.8	90.2	0.0	204	—	—	—	—
Better type (23)	91.1	8.9	0.0	1943	—	—	—	—
Intermediate type (19)	94.2	5.8	0.0	1591	—	—	—	—
A 4278**	92.9	7.1	0.0	85	—	—	—	—
A 42441 (593)**	79.3	20.7	0.0	87	—	—	—	—
Progeny of normal plants of 42163								
44271	9.2	90.8	0.0	284	16.7	83.3	0.0	60
44273	3.8	96.2	0.0	26	0.0	100.0	0.0	16
44275	18.7	81.3	0.0	241	8.7	91.3	0.0	23
44277	33.9	66.1	0.0	407	19.2	80.8	0.0	73
44281	8.3	91.7	0.0	532	1.6	98.4	0.0	123
44283	74.3	25.7	0.0	338	54.1	45.9	0.0	85
44284	13.8	86.2	0.0	672	2.2	97.8	0.0	45
44285	15.2	84.8	0.0	741	7.1	92.9	0.0	99
Total	21.9	78.1	0.0	3241	15.6	84.4	0.0	524
Progeny of aberrant plants of 42163								
44272	0.0	0.0	100.0	6	0.0	0.0	100.0	1
44274	0.0	0.0	100.0	17	0.0	0.0	100.0	7
44276	0.0	0.0	100.0	3	0.0	0.0	100.0	1
44280*	0.0	32.0	68.0	75	1.6	13.6	84.8	191
44282*	0.0	16.7	83.3	66	1.1	15.6	83.3	179
44286*	2.0	22.6	75.4	199	2.1	18.9	79.0	391
Total	1.1	21.9	77.0	366	1.7	16.8	81.5	770

The following information may be obtained by examining the data of table 1 given under the subheading "Collection from 02 Ranch in Texas and variety 593." Ninety and two-tenths percent of the plants grown from seed collection 42163 were aberrant. The seed collected from the other 19 intermediate-type plants produced on an average only 5.8 percent aberrant plants, the seed from

the 23 better-type guayule plants 8.9 percent, the non-selected seed collection 7.1 percent, and the seed of the commercial variety 20.7 percent. It is evident from these data that the plant from which seed collection 42163 was taken is unusual because of the extremely high proportion of aberrant to normal plants in its progeny. Since STEBBINS and KODANI (1944) and POWERS and ROLLINS (1945) have shown that these aberrant plants arise from 72±-chromosome plants as a result of non-reduction followed by fertilization, it seems that the plant from which seed collection 42163 was taken must have been an abnormal facultative amphimict (predominantly non-reduction in number of chromosomes followed by fertilization) or an aberrant facultative apomict. In case the latter supposition is correct, the normal plants could have arisen as a result of reduced apomixis (reduction followed by failure of fertilization) and would be expected to have 54± chromosomes. Chromosome counts of these normal plants showed that such was not the case, the chromosome number being 72±. If the first supposition is correct, these normal plants should produce a high proportion of aberrant plants, and the aberrant plants should produce a correspondingly high proportion of plants with more than 108± chromosomes.

The data for the progeny of normal plants of 42163 and that for the progeny of aberrant plants of 42163 are given in table 1, also. The data show that for both years the normal plants on an average gave a preponderance of aberrant plants, 78.1 percent in 1943 and 84.4 percent in 1944. However, the Chi-square test showed that these eight strains do not form a homogeneous group as regards the proportion of normal to aberrant plants. The progeny of plant 44283 has a higher proportion of normal than aberrant plants for both years, while the reverse is true for the other seven plants. Thus it can be seen that seven of these eight normal plants are producing normals and aberrants in the ratio that would be expected if they and the original plant from which they came are abnormal facultative amphimicts—that is, the majority of their progeny rise as a result of non-reduction followed by fertilization. All eight of these typical normal plants were found to have 73 chromosomes including one small one.

The data for the progeny of aberrant plants derived from seed collection 42163 reveal that for both years the proportion of hyperaberrant plants was greater than the proportion of aberrant plants. Furthermore, the percentage of hyperaberrant plants in the progeny of aberrant plants closely approximates the percentage of aberrant plants in the progeny of the normal plants. This is true of the data for both years, the percentages being 77.0 and 78.1 respectively for 1943, and 81.5 and 84.4 respectively for 1944. If these hyperaberrant plants have a higher chromosome number than the aberrants, it is evident that these data also confirm the supposition that the original plant from which seed collection 42163 was taken is an abnormal facultative amphimict, and further it may be concluded that probably the majority of the aberrant progeny of this plant are also abnormal facultative amphimicts.

However, to substantiate this later conclusion, it is necessary to know whether or not the majority of the aberrant plants produce progeny and

whether or not the plants classified could be taken as representative of those that emerged. In 1943 the number of seeds produced per aberrant plant was small; consequently only three progenies from individual plants were grown. Three additional progenies involving all the seed of aberrant plants growing in three different locations were grown also. In 1944 the seed of aberrants was planted on the basis of individual plants, and the data were recorded on the basis of individual plant progenies. The number of individual plant progenies was 173. This number was too great to report the data on the basis of individual plant progenies since very little additional information would be gained by so doing. This is especially true, since only 0.89 percent, on an average, of the 500 seeds planted per progeny produced seedlings. Consequently the 1944 data for the progeny of aberrants are reported on the same basis in table 1 as are the 1943 data. But, as previously pointed out, these data need to be supplemented with the following information taken from the individual plant progenies. Only 24 of these 173 aberrant plants failed to produce offspring. The plants were classified at the time the normal plants in the study had from three to five true leaves. At this time the percentage of plants of any culture that had died after emergence was so small as to be negligible. This fact was established by comparison with emergence counts taken every three days for 21 days. At each time counts were made any dead plants were noted and included as having emerged. They were also marked with a wooden applicator so as to be properly recorded in later determinations. Also, at this time it should be noted that all plants which survived were grown until the normal plants had begun to bloom. This allowed the earlier classification to be substantiated. With these facts in mind, it is evident that the data presented in table 1 for the progeny of the aberrant plants are representative, because a majority of them produced progeny and because so few plants died between emergence and classification as to be of no significance. Although it will not be pointed out later, data which follow also substantiate this conclusion.

This behavior of the progenies of the normal and the aberrant plants recorded in table 1 may be taken as evidence tending to substantiate the conclusion that the original plant from which seed collection 42163 was taken is an abnormal facultative amphimict. Certain other problems arise in connection with this study which need to be solved in order to obtain definite proof of the above conclusion and in order to learn more about the reproduction processes operating in *Parthenium argentatum*. It is necessary to know the effect of different sources of pollen upon the ratio of normal to aberrant plants. Also, the supposition that the aberrants arising from normal plants and the hyperaberrants arising from the aberrant plants result from non-reduction followed by fertilization needs to be substantiated for this material. A determination of the chromosome numbers of the progenies will be used to do this.

The Effect of Different Sources of Pollen upon the Percentages of Normal and Aberrant Plants in the Progeny of Normal Plants of Seed Collection 42163

The data concerning the effect of different sources of pollen upon the percentages of normal and aberrant plants in the progeny of normal plants of

seed collection 42163 are given in table 2. Six normal plants were included in the study, all of which had 73 chromosomes. The treatments were as follows: cross-pollination, self-pollination, and non-controlled pollination. In the cross-pollinated treatment 7422-59 was pollinated with pollen from plants having 36+ chromosomes, 7423-1 with pollen from plants having 54± chromosomes, and 7423-24 with pollen from plants having 72± chromosomes. From the data in the table it may be seen that the proportion of aberrant plants in-

TABLE 2

The effect of different sources of pollen upon the percentages of normal and aberrant plants in the progeny of normal plants.

POLLINATION AND PLANT CULTURE	NORMALS	ABERRANTS	PLANTS CLASSIFIED
	Percentage	Percentage	Number
Cross-pollinated			
7422-59×plants with 36+ chromosomes	20.0	80.0	60
7423- 1×plants with 54± chromosomes	8.3	91.7	24
7423-24×plants with 72± chromosomes	4.9	95.1	122
Average percent	9.7	91.3	—
Total number	20.0	186.0	206
Self-pollinated			
7422-71	16.3	84.7	59
7423-68	5.0	95.0	100
7423-50	0.0	100.0	40
Average percent	7.0	93.0	—
Total number	14.0	185.0	199
Pollen not controlled			
7422-71	11.5	88.5	52
7423-68	21.8	78.2	110
7423-50	3.8	96.2	52
Average percent	15.0	85.0	—
Total number	32.0	182.0	214

creases with an increase in the chromosome number of the pollen parent. The χ^2 test applied to the original numbers showed that the difference between 7422-59 and 7423-24 in percentage of aberrant plants is statistically significant. Since the same seed plants were used in the self-pollinations and the non-controlled pollinations, the data for these two treatments are directly comparable. The progenies resulting from self-pollinations averaged significantly higher in the percentage of aberrant plants. Again Chi-square shows that this difference cannot be attributed to chance. These data rather conclusively prove that the source of pollen does have an effect upon the ratio of normal to aberrant plants. However, the important fact is that the method of

reproduction of these plants has not been changed, since all of them, regardless of the source of pollen, produce a considerably higher percentage of aberrant than normal plants.

Determination of Chromosome Numbers of the Progenies

Obviously the chromosome numbers of all the plants of all the progenies could not be determined because of the magnitude of such a task. Therefore it was decided to make chromosome number counts in root tip cells of an adequate number of seedlings to determine the usual behavior (whether non-reduction was being followed by fertilization) of the material, and to accomplish the same on a more extensive scale by measuring the diameter of pollen grains of individual plants of certain progenies. That chromosome number and diameter of pollen grains are associated in guayule is shown by BERGNER (unpublished data).

Root Tip Counts

The three plants whose progeny were selected for root tip counts were growing in culture 3562. This culture was composed of 12 plants, planted in a 3×4 plot in which the space between plants was four feet each way. The three plants used in the study were growing in tandem in the first row of the plot and were 3562-1, 3562-2, and 3562-3. The seed taken from each of these plants for chromosome number counts resulted from non-controlled pollinations. Plant 3562-1 was an aberrant plant with a height of 19 centimeters and a spread of 34 centimeters. Plants 3562-2 and 3562-3 were typical normals and had heights and spreads expressed in centimeters of 31 and 66, and 32 and 65, respectively. These latter two plants were phenotypically non-distinguishable. In progeny tests, 3562-1, from 500 seeds, produced 20 plants of which 5 percent was aberrant and 95 percent was hyperaberrants; 3562-2, from 1000 seeds, produced 177 plants of which 63 percent was normal and 37 percent was aberrant; 3562-3, from 1000 seeds, produced 305 plants of which three percent was normal and 97 percent was aberrant. The percentages of the seeds of these three plants which produced seedlings should be given now because of the bearing they might have upon the interpretation of these ratios. Four percent of the seeds of 3562-1 produced seedlings. The seed resulted from non-controlled pollinations. Nineteen percent of the seed of 3562-2, resulting from non-controlled pollinations, produced seedlings that emerged in 1943 and 17.7 percent of the seed produced seedlings in 1944. The corresponding figures for 3562-3 were 30 and 31 percent, respectively. In 1943 these same plants were self-pollinated. The percentages of the seed which produced seedlings were 28 for seed from 3562-2 and 56 for seed from 3562-3. This shows that the percentages of seeds that produce seedlings is greater for the normal plant producing a higher percentage of aberrants than normals than it is for the normal plant producing a higher percentage of normals than aberrants. This proves that the differential ratios obtained could not be due to a greater loss of aberrant seedlings before emergence. Seventeen percent of the seed of 593 from non-controlled pollinations produced seedlings in 1943, and 29 percent from self-pollinations in 1943. Hence, it is apparent that the percentage emer-

gence for seed from these two normal plants derived from collection 42163 is at least as high as that of the best and most extensively grown commercial variety. This is also true of the other normal plants derived from 42163 and reported on in this article.

The above information concerning these three plants makes it clear that a study of the chromosome numbers of a few plants of each progeny should aid materially in establishing the chromosome numbers of the normal plants, the aberrant plants, and the hyperaberrant plants derived from seed collection 42163. If the assumption is correct that in the case of 3562-1 the hyperaberrant plants arise as a result of non-reduction followed by fertilization, then the majority of the progeny of 3562-1 should have more than 108± chromosomes, the exact number depending upon the chromosome number of the pollen bringing about fertilization. Root tip counts were made of three different seedlings which were found to have 137, 142 and 144 chromosomes. Since the majority of the progeny of 3562-2 have been shown to be normals, the majority of the seedlings grown from seed of 3562-2 should have 72± chromosomes. Of five seedlings whose chromosomes were counted, four had 72± chromosomes and one had 108± chromosomes. The majority of the progeny of 3562-3 would be expected to have 108± chromosomes, since this plant was shown to produce a high proportion of aberrant offspring. Three of the five seedlings whose chromosome number was counted had 108± chromosomes, one had 99± chromosomes, and one had 78± chromosomes. Clearly these findings furnish rather convincing evidence that the normal plants of this material have 72± chromosomes, the aberrant plants from 90± to 108± chromosomes, depending upon the source of pollen, and the hyperaberrant plants have more than 108± chromosomes.

Pollen Diameter Measurements

Two types of pollen diameter measurements were taken. One set of measurements was taken from base of spines on exine to base of spines on exine; whereas, the other set was taken from inside wall of intine to inside wall of intine. Also, to determine the effect upon diameter of pollen grains of the different environments ordinarily encountered in field experiments, measurements were taken on eight plants of each of six different replicates.

The means, standard errors, and variances are given in table 3 for diameter of pollen grains and number of pollen grains measured for plants of different chromosome number groups and plants of the same chromosome group but of different replicates. From this table it may be seen that the chromosome number groups occur in multiples of nine. In every case an increase in chromosome number has resulted in an increase in the mean diameter of the pollen grains and in the size of the variances with the exception of the variance comparison involving the 54±- and the 72±-chromosome plants and that involving the 81±- and 90±-chromosome plants. Considering differences due to replicates, it may be seen that there are no significant differences between mean diameters of pollen grains for the first four replicates. However, the mean diameters of pollen grains for the last two replicates are significantly larger than the mean diameters for the first four replicates, clearly showing that the

environment has an effect upon size of pollen. Hence, caution must be exercised in making pollen-size comparisons between materials not growing in the same experiment or where the environmental influences have not been conrolled otherwise.

TABLE 3

The means, standard errors, and variances for diameter of pollen grains and number of pollen grains measured for plants of different chromsome number groups and plants of the same chromosome group but of different replications. One hundred pollen grains were measured per plant.

CHROMOSOME GROUP OR REPLICATION	MEASUREMENTS FROM BASE OF SPINES ON EXINE TO BASE OF SPINES ON EXINE			MEASUREMENTS FROM INSIDE WALL OF INTINE TO INSIDE WALL OF INTINE		
	MEAN AND STANDARD ERROR	VARIANCE	POLLEN GRAINS MEASURED	MEAN AND STANDARD ERROR	VARIANCE	POLLEN GRAINS MEASURED
	Microns	Microns	Number	Microns	Microns	Number
Chromosome group						
36+	22.48±0.101	1.960	1000	15.34±0.116	1.234	2600
54±	25.89± .127	2.576	1000	17.06± .213	2.411	1400
72±	26.58± .123	2.511	1000	18.76± .197	2.607	2200
81±	—	—	—	19.98± .334	3.190	900
90±	—	—	—	21.13± .218	2.713	300
108±	29.67± .219	6.031	1000	22.18± .193	3.826	1800
Replication (108± chromosomes)						
3107	30.21±0.332	7.587	800			
3562	30.15± .311	6.277	800			
3798	30.02± .183	5.773	800			
4121	30.27± .273	5.851	800			
4600	31.22± .271	4.762	800			
5101	31.36± .460	6.778	800			

The means, standard errors, and variances for diameter of pollen grains for five different strains are given in table 4. The normal progeny of 44281, 44283, and 44271 averaged 26.87, 26.77, and 26.58 microns in diameter, whereas the aberrant plants from these same strains averaged 29.93, 29.57, and 29.67 microns in diameter. It is evident that the normal plants of these strains belong to the 72±-chromosome group and the aberrant plants to the 108±-chromosome group. The aberrant plants of 44282 and 44286 averaged 29.63 and 29.58 microns in diameter respectively; whereas, the hyperaberrant plants of these two strains averaged 31.65 and 31.52 diameters in microns, respectively. These data show that regardless of which strain they occur in there are no statistically significant differences between the mean diameters of the pollen grains of the normal plants, of the aberrant plants, nor of the hyperaberrant plants; but the mean diameters of any one of these groups are significantly different from those of any other.

Weight Per Flowering Branch

In other studies in which chromosome counts had been made it was noted that the flowering branch including the capitula was definitely larger for the 108±-chromosome group than for the 72±-chromosome group. Hence, weight per flowering branch could be used as indicating increases or decreases in chromosome number. These data are given in table 4. Again, as in the case of

TABLE 4

Means, standard errors, and variances for diameter of pollen grain and for weight of flowering branch of strains. The plants were classified (within strains) as normal, aberrant, and hyperaberrant. The type of the seed parent is given in the last column of the table.

STRAIN	DIAMETER OF POLLEN GRAINS		WEIGHT OF FLOWERING BRANCH		TYPE OF SEED PARENT
	MEAN	VARIANCE	MEAN	VARIANCE	
	Microns	Microns	Grams	Grams	
			Normal		
44281	26.87±0.117	2.068	0.409±0.0241	0.005810	Normal
44283	26.77± .110	2.657	.399± .0381	.014499	Normal
44271	26.58± .123	2.511	.384± .0407	.016538	Normal
			Aberrant		
44281	29.93±0.230	5.867	0.581± .0456	0.020788	Normal
44282	29.63± .558	3.541	.670± .0950	.027100	Aberrant
44283	29.57± .261	5.511	.638± .0520	.027062	Normal
44286	29.58± .401	6.070	.651± .0632	.039921	Aberrant
44271	29.67± .219	6.031	.567± .0342	.011712	Normal
			Hyperaberrant		
44282	31.65±0.843	9.539	0.833±0.0338	0.003434	Aberrant
44286	31.52± .306	9.333	.741± .0752	.056477	Aberrant

mean diameters of pollen grains, there are no significant differences between means within any given type, regardless of strain; but there are significant differences between types—the weight per flowering branch is greatest for the hyperaberrants, less for the aberrants, and least for the normals.

The data on root tip chromosome counts, measurements of diameters of pollen grains, and weights per flowering branch are conclusive in showing that in general the aberrants belong to a higher chromosome number group than the normals and the hyperaberrants to a higher chromosome number group than the aberrants. Then, it follows that as a rule the aberrants arise from the normals and the hyperaberrants from the aberrants as a result of non-reduction followed by fertilization. Such being the case, the data given in the foregoing four tables definitely prove that the plant growing on the 02 Ranch in Texas from which seed collection 42163 was taken is an abnormal facultative amphimict.

EXPERIMENTAL RESULTS PERTAINING TO THE OCCURRENCE OF METHODS OF REPRODUCTION OTHER THAN ABNORMAL FACULTATIVE AMPHIMIXIS

The normal plants among the first generation progeny of this unusual Texas plant could have arisen as a result of normal sexuality (reduction followed by fertilization), segregating apomixis, non-segregating apomixis, or some combination of the three. The latter supposition seems highly probable, since POWERS and ROLLINS (1945) in a study of the methods of reproduction occurring in guayule found that normal sexuality, non-segregating apomixis, and probably diplospory could be occurring in a single plant. The balance of the experimental data pertains to methods of reproduction occurring in this material other than abnormal facultative amphimixis.

Variation in Collections from the Natural Population in Which the Plant Giving Rise to Seed Collection 42163 Was Growing

The greater part of the natural population growing on the 02 Ranch in Texas was composed of the better-type guayule plants. In fact, 89.5 percent of the non-selected sample taken from this population was of the better type. The percentages of aberrant plants in the populations grown from the different seed collections made from plants growing on the 02 Ranch have been given previously and need not be repeated here. For the different populations the percentage of normal plants that were off-type are as follows: 41.2 for 42163, 0.7 for the 19 collections from intermediate-type plants, 3.3 for the 23 collections from the better morphological type plants, 1.3 for the non-selected sample, and 5.8 for variety 593. Clearly, there is a correspondingly higher-proportion of morphologically distinguishable plants among the normals grown from seed collection 42163. In fact, seven of the 18 normal plants grown from this collection were not of the general intermediate type. The remaining 11 plants were not morphologically distinguishable from each other nor could they be distinguished morphologically from the typical normal plants of the 19 intermediate-type populations. Nine of these normal plants behaved alike in that they produced a higher proportion of aberrants than normals. However, the remaining two of these 11 plants produced a higher proportion of normals than aberrants. If some of the normal plants derived from seed colection 42163 are producing more normal than aberrant plants, then some of the aberrants derived from this same collection would be expected to be producing more aberrant than hyperaberrant plants This was true of ten of the 149 aberrant plants that produced progeny. From these results it is evident that at least some of the normal plants derived from normals and some of the aberrant plants derived from aberrants are produced by some method of reproduction that permits segregation.

Phenotypic Variability between Strains

ROLLINS (1944) has shown that the intermediate-type plants of guayule are of hybrid origin, mariola (*P. incanum* (Gray)) having played a part in their production. POWERS and ROLLINS (1945) have shown that these intermediate-type plants are facultative apomicts and that those members of the progeny resulting from normal sexuality are readily distinguishable morpho-

logically from those resulting from some form of non-segregating apomixis. Hence, it seems safe to conclude that these intermediate-type plants are highly heterozygous. Such being the case, the degree and type of variation occurring should furnish some information as to the method of reproduction occurring in the material. For example, the plants resulting from normal-sexual reproduction should differ materially from each other, both as to morphological and physiological characteristics. On the other hand, plants originating by some means of non-segregating apomixis and grown under similar environments should not differ significantly from each other as to morphological or physiological characteristics. This would be particularly true of comparisons between the progenies of such plants. Diplospory represents a method of reproduction intermediate between normal sexuality and non-segregating apomixis. (In this article diplospory is used to denote that method of reproduction in which semi-heterotypic divisions (ROSENBERG 1926–27) and restitution nuclei occur (with or without dyad formation).) The work of GUSTAFSSON (1939) shows that numerous transitional stages between meiosis and sexual mitosis occur in facultative-apomictic species. Then, it follows that different degrees of chromosome pairing may take place during the semi-heterotypic division. That is, synapsis may vary in degree so that the number of pairs of chromosomes that have undergone synapsis may vary from one pair to all the pairs. The result is that the genic segregation may or may not be great. It should be noted that this use of the term semi-heterotypic divisions follows STEBBINS (1941). It is evident that diplospory could allow for a limited amount of segregation or considerable segregation, depending upon the degree of pairing and genic crossing over that is taking place.

Five progenies from as many strains were used to study the phenotypic variability between strains. The progenies of strains 44281, 44283, and 44271 were from individual plants and those of 44282 and 44286 came from aberrant plants whose seed had been bulked to produce a larger sample, there being seed from 50 different plants in each bulked sample. The pollen giving rise to the seed of these progenies was not controlled. Five quantitative characters were studied in all of these strains and four additional characters in strains 44281, 44283, and 44271.

The data and characters studied are given in tables 4 and 5. From table 4 it may be seen that there are no differences between strains within the normals, within the aberrants, or within the hyperaberrants as regards mean diameter of pollen grains or weight of flowering branch. The data of table 5 show that the same is true for height of plant, spread of plant, number of microcytes, length of leaf, and length of petiole. The differences in width of leaf between 44281 and 44283, and 44281 and 44271, are statistically significant, if the data for width of leaf are considered alone. The same is true for the difference in number of leaf lobes between 44271 and 44283. However, considering the data as a whole, the number of comparisons made is great, making it probable that differences as great as these could have occurred by chance.

These quantitative data confirm the evidence from a rather intensive study of the morphological characters—namely, that the plants of any given strain, as a whole, are very similar to those of any other strain. To a considerable

extent this is also true of the typical normal plants within strains but is not true of the aberrant or hyperaberrant plants. The within strain differences of aberrants are rather great for aberrants and still more marked for hyperaberrants.

The progenies from strains 44281, 44283, and 44271 are so similar that one might suspect that the three normal plants giving rise to these progenies arose from the original plant on the 02 Ranch in Texas as a result of non-segregating apomixis. That such was not the case is shown by the fact that the majority

TABLE 5

Means and standard errors for the designated plant characters.

STRAIN	HEIGHT OF PLANT	SPREAD OF PLANT	MICROCYTES	LENGTH OF LEAF	LENGTH OF PETIOLE	WIDTH OF LEAF	LEAF LOBES
	Centimeters	Centimeters	Number	Centimeters	Centimeters	Centimeters	Number
			Normals				
44281	25.06±0.528	23.31±0.644	9.6±1.301	6.4±0.126	2.2±0.038	0.730±0.017	5.5±0.086
44283	25.12± .645	22.95± .408	11.6±1.108	6.4± .110	2.2± .075	.821± .022	5.6± .089
44271	25.00± .665	22.94± .543	12.4±2.109	6.2± .092	2.1± .062	.806± .016	5.3± .073
			Aberrants				
44281	17.81±0.971	16.38±0.939	14.4±1.802				
44282	19.00±3.215	19.00±1.528	6.7±2.728				
44283	18.31±0.939	16.25±0.710	13.3±1.126				
44286	21.50±1.174	20.00± .558	9.8±1.849				
44271	19.56±0.707	17.06± .413	11.4±1.945				
			Hyperaberrants				
44282	14.67±1.856	15.00±1.528	11.7±1.764				
44286	14.10±0.482	14.80±0.573	12.2±2.284				

of the plants of progeny 44283 were normal, whereas the majority of the plants of the progenies 44281 and 44271 were aberrants.

To test further the variation among the normal plants derived from the individual plant strains 44281, 44283, and 44271, ten normal plants were taken at random from each of these strains for further tests. These plants did not show any morphological differences between plants of the same strain or between plants of different strains. The data are given in table 6 for percentage of aberrant plants and for percentage of seedlings that emerged. It is well to keep in mind that these data are for the third generation. As was the case in the first and second generations, the abnormal facultative amphimicts produced a predominance of abnormal-facultative-amphimictic offspring, there being nine such plants for strain 44281 and eight such plants for strain 44271. It will be remembered that the plant which gave rise to strain 44283 was a facultative apomict. Nine of the ten plants derived from it were again facultative apomicts. The remaining plant number 10 proved to be an abnormal facultative amphimict. This plant in respect to method of reproduction is behaving like its grandparent, the plant growing on the 02 Ranch in Texas from which seed collection 42163 was taken. Thus the cycle from abnormal facultative amphimixis to facultative apomixis back to abnormal facultative amphimixis has been completed.

Turning to a consideration of the percentage of seedlings that emerged,

it may be seen that the percentage of seeds which produced seedlings was higher for the two abnormal facultative amphimicts than it was for the facultative apomict—that is, 44281 and 44271 compared with 44283. It will be remembered that the seed of 3562-2, a facultative apomict, gave a lower percentage of seedlings from 1000 seeds than did plant 3562-3, an abnormal facultative amphimict. The most striking illustration of this association between method of reproduction and percentage of seed which produces seedlings

TABLE 6

Percentages of aberrant plants and number and percent of seedlings that were obtained from 500 seeds of each of ten normal plants of each of the following: 44281, 44283, and 44271.

PLANT NUMBER	ABERRANT PLANTS			SEEDLINGS					
	44281	44283	44271	44281		44283		44271	
	Percentage	Percentage	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
1	86.2	13.7	81.5	174	34.8	124	24.8	157	31.4
2	93.4	22.1	13.5	241	48.2	149	29.8	52	10.4
3	92.8	7.6	83.0	235	47.0	145	29.0	188	37.6
4	74.1	15.6	96.9	201	40.2	141	28.2	160	32.0
5	91.3	7.5	7.5	161	32.2	133	26.6	146	29.2
6	35.2	10.9	70.9	142	28.4	128	25.6	196	39.2
7	71.1	7.4	98.1	201	40.2	163	32.6	106	21.2
8	100.0	26.4	78.1	31	6.2	163	32.6	178	35.6
9	80.4	37.0	89.9	97	19.4	127	25.4	208	41.6
10	97.4	71.3	77.5	231	46.2	209	41.8	209	41.8
Total	82.6	24.2	74.2	1714	34.3	1482	29.6	1600	32.0

is furnished by plant 10 of strain 44283. A return to abnormal facultative amphimixis in this plant was accompanied by an increase in the percentage of the seed that produced seedlings. Of further interest is the fact that plants 8 and 9 strain 44281 and plants 2 and 7 strain 44271 are definitely lower in percentage of seed that produced seedlings than are the other plants of the same strain. Considering both the percentage of aberrant plants and percentage of seedlings that were obtained from 500 seeds of each plant, it is apparent that at least three of the plants of 44281, one of the plants of 44283, and two of the plants of 44271 are behaving differently than the parents from which they were derived. That these occur so frequently shows that the number of gene-pairs differentiating fertilization of the egg-cell from non-fertilization of the egg-cell cannot be great. Furthermore, since 44281, 44283, 44271, and in fact all the first generation typical-normal plants were found by DR. BERGNER to have 73 chromosomes, these changes must be genic rather than one of chromosome number.

The great uniformity of these normal plants in respect to morphological characters and the physiological characters given in tables 4 and 5, together with the fact that all the typical-normal plants of the first generation were found to have 73 chromosomes, rather definitely proves that normal sexuality

is not the sole method of reproduction by which they are produced. Yet the progeny tests, involving three generations, show that these normal plants are segregating both for methods of reproduction and percentage of seeds that produce viable seedlings. Consequently, all these plants have not resulted from some form of non-segregating apomixis. The limited amount of segregation and the fact that all these typical-normal plants have 73 chromosomes, regardless of whether their prevailing method of reproduction is non-reduction followed by fertilization or non-reduction followed by failure of fertilization, proves rather conclusively that diplospory as previously defined in this article is occurring in this material. Some of the off-type normals with $72\pm$ chromosomes show evidence of having come about by fertilization with pollen of mariola and in other cases with pollen of the better guayule type plants. This is rather conclusive proof that the method of reproduction is facultative (normal sexuality occurs to some extent), as was found to be the case for other strains of guayule by POWERS and ROLLINS (1945). Also, POWERS and ROLLINS (1945) found that non-segregating facultative apomixis was the prevailing method of reproduction in some strains. These facts show that in guayule the methods of reproduction are not distinct but that they grade into each other. Abnormal amphimixis (non-reduction followed by fertilization), diplospory (semi-heterotypic division followed by restitution nuclei, but not necessarily dyad formation, and by failure of fertilization), and non-segregating apomixis (somatic type of division (see GUSTAFSSON 1939) followed by failure of fertilization) probably are occuring in single plants in these populations derived from seed collection 42163. This conclusion is consistent with both the findings of GUSTAFSSON (1939) and the conclusions of STEBBINS (1941).

DISCUSSION PRESENTING A HYPOTHESIS AS TO THE EVOLUTION OF APOMIXIS AND POLYPLOIDY

In considering the evolution of apomixis and polyploidy, only a few of the papers having a bearing on the subject need be mentioned. For more extensive reviews the reader is referred to GUSTAFSSON (1939) and STEBBINS (1941).

BABCOCK and STEBBINS (1938) state that in dealing with agamic complexes there is no doubt that the apomicts have been derived from the sexual members of the complex and that in all agamic complexes the obligate apomicts are probably preceded by the facultative ones in the development of the complex. That there is a very definite correlation between apomixis and polyploidy has been pointed out by a number of workers (review STEBBINS 1941). However, as pointed out by STEBBINS, this association is not absolute, since diploid apomictic species are known and sexual polyploids exist in a number of genera, some of the members of which are apomicts. Also, the investigations of the various workers have shown that the two major processes involving normal sexuality are independent to a considerable extent. That is, reduction in chromosome number does not assure that fertilization will take place. Likewise, failure of reduction in chromosome number is not necessarily accompanied by failure of fertilization.

If all forms of apomixis are considered at one time, the primary processes become confused. Therefore, emphasis will be placed on pseudogamous di-

plospory, because probably it precedes some if not all other types of apomixis in the development of agamic complexes. To understand the inheritance of apomixis, it is necessary to appreciate that major processes are involved, and that these are independent of each other, at least to some extent. The three major steps involving the change from normal sexuality to pseudogamous diplospory are (1) failure of reduction of number of chromosomes, (2) failure of fertilization, (3) development of the egg-cell into a new individual without its being fertilized.

Some information is available concerning the inheritance of these steps in plants. SATINA and BLAKESLEE (1935) report a case in Datura in which normal sexuality is differentiated from failure of reduction by one factor pair. Failure of reduction was recessive, and a small percentage (about 1 to 5 percent) of megasporocytes and microsporocytes in dyad plants developed normally. SATINA and BLAKESLEE make the following statement concerning this recessive gene. "In the gene 'dyad' which has been described here, it is the failure of the second meiotic division which leads to the formation of a diploid number of chromosomes in the gametes. The exact mechanism by which the gene suppresses the second division is not known, but the effect appears to be brought about through a premature cell wall formation which anticipates the second division, or, perhaps more likely, through a great extension of the telophase stage which in normal plants is of brief duration. Whatever may be the immediate effect of the gene by means of which it brings about dyad formation, its action is influenced by environmental factors, as shown by the exceptional cases of tetrad formation." Plainly, failure of reduction in this case is a quantitative character, one of degree and is differentiated by one recessive factor pair.

The data of this present article provide some genetic evidence concerning the manner of inheritance of genes differentiating failure of fertilization from the normal process. Again, the normal process (fertilization) seems to be dominant but not completely so; this shows the quantitative nature of this process. The number of major gene pairs differentiating the fertilization process in this material, as shown previously, must be comparatively few, probably at most not more than two major gene pairs.

Not much information is available concerning the manner of inheritance of the stimulation which causes the development of the egg-cell. It seems that the development of the egg-cell into an embryo is independent, at least in part, from failure of reduction and failure of fertilization. This is shown by the following quotation from GUSTAFSSON (1935). "The problem of the development of the egg-cell is somewhat different. In the parthenogenetic apomicts a distinction must be made between the processes by which the progenies of the E.M.C.'s acquire the zygoid chromosome number and the stimulation which causes the development of the egg-cell. That this distinction is correct is shown among other things, by the cases in which the zygoid embryo-sacs, whether parthenogenetic or aposporous, of *Gastrodia elata* (KUSANO 1915), *Artemisia nitida* (CHIARUGI 1926). *Ochna serrulata* (CHIARUGI and FRANCINI 1930) do not contain any egg-cells capable of development in spite of their being quite normal. Multiplication takes place instead in the first case by means of normal

fertilisation, in the second by rhizomes, in the third by adventitious embryos. Neither in sexual species, in which the egg-cell for some reason or other has obtained the zygoid number, does it develop without fertilisation." This shows that the stimulation to develop or capacity of an egg-cell to develop into a functional embryo without having been fertilized is independent of failure of reduction and failure of fertilization.

At this time certain other facts and considerations need to be mentioned. GUSTAFSSON (1939) points out that unreduced egg-cells develop as a result of semi-heterotypic divisions followed by the formation of restitution nuclei, as a result of pseudohomeotypic divisions, and as a result of so-called somatic divisions. Numerous gradations between these occur. Then, the semi-heterotypic divisions and the pseudohomeotypic divisions are overlapping processes in the sense that both phenomena can and probably do occur in the same individual and in the sense that synapsis may take place in all the chromosome pairs, most but not all of the chromosome pairs, few of the chromosome pairs, or none of the chromosome pairs. Even though the change is gradual from the semi-heterotypic division to the pseudohomeotypic division, if any pairing occurs it will be considered a semi-heterotypic division, and if no synapsis of chromosomes occur as a pseudohomeotypic division (see STEBBINS 1941). Also, as was done by GUSTAFSSON (1939), both the semi-heterotypic and pseudohomeotypic divisions will be classed under diplospory. This does not imply that dyads are formed, because, as GUSTAFSSON points out, in some Hieracium a direct transition to binuclear embryo-sacs is the most common. By this use of the terms, semi-heterotypic division and pseudohomeotypic division have very definite meanings, since segregation of genes can occur in the case of the former but not in the case of the latter. Since, as has been shown by a progeny test, a limited amount of segregation occurs as regards the normal progeny of 44281, 44283, and 44271, that type of diplospory involving the semi-heterotypic division and restitution nuclei must be prevalent in this material, and furthermore the number of chromosomes that pair must be few because of the limited amount of segregation. In this article the term hybrid is used in the genetic sense—that is, to denote an individual that arises as a result of the union of two genetically dissimilar gametes which came from two individuals, and these two individuals may be members of the same or different species. Interspecific hybridization may or may not have been involved in the development of apomixis and polyploidy in *Parthenium argentatum*. Whether it was or was not is immaterial as regards this discussion, because the fundamental considerations would be the same. That hybridization as defined must have played a part will be shown. In developing the hypothesis as to the origin of polyploidy and apomixis, only diplospory in which the semi-heterotypic divisions and restitution nuclei are occurring will be considered, since variations based on known processes would explain the origin of the other types of apomixis. Also, in considering the evolution of polyploidy and apomixis in Parthenium it must be kept in mind that we are dealing with facultative processes and these processes are quantitative characters, ones of degree. This is important, since it allows for the evolution of apomixis by gradual steps.

For the sake of clarity of explanation each major step in the evolution of apomixis and polyploidy will be considered as differentiated by a single gene pair, even though this may be far more simple than actually is the case. Then, the heterozygote for each of the three major steps designated previously may be symbolized as follows:

(1) *Aa* are the alleles differentiating reduction in number of chromosomes as opposed to failure of reduction.

(2) *Bb* are the alleles differentiating fertilization of egg-cell as opposed to failure of fertilization of egg-cell.

(3) *Cc* are the alleles differentiating non-development of egg-cell into embryo without its being fertilized as opposed to development of egg-cell into embryo without its being fertilized.

Further, assume that the apomictic forms arose from the sexuals. To date, all of the strictly sexual forms examined have 36+ chromosomes, which is the lowest number of chromosomes known to occur in *Parthenium argentatum*. Although 1n-chromosome plants have been found among the progeny of 72±-chromosome plants, no 1n individuals have been found among the progeny of the 36+-chromosome plants. This is rather convincing evidence that the 36+-chromosome group represents the 2× condition, and therefore the ancestors of the present 36+-chromosome plants were also the ancestors of the polyploid forms. That is, the ancestors of the polyploid forms had 36+ chromosomes. Then, starting with this information, a highy plausible hypothesis may be developed for the explanation of the evolution of polyploidy and apomixis in Parthenium.

Using the above genotypic symbols, the genotype of the 36+-chromosome homozygous dominant sexual would be *AABBCC*, and the genotype of the 54±-chromosome facultative apomict that carried the greatest possible number of recessive genes would be *aaabbbccc*. The problem has become one of determining how the latter genotype arose from the former. Which of these genes *A*, *B*, or *C* mutated to the recessive first is immaterial to the present considerations because all of these recessives could be carried in the heterozygous condition and in the homozygous condition, since the processes controlled are facultative and not obligate. For the sake of clarity it is well to pursue this contention farther by considering the behavior of different genotypes.

The majority of the progeny from the genotype *aaBBCC* would be triploids because the *aa* genes would cause the majority of the egg-cells formed from the egg-mother-cells to be non-reduced, and fertilization would increase the chromosome number to 54±. However, since non-reduction is facultative, *aaBBCC* individuals would be produced and could maintain the genotype in the natural populations. The majority of the egg-cells of the triploids would again be non-reduced, and fertilization would again increase the chromosome number. Consequently, the genotype *aaBBCC* could not give rise to a stable 54±-chromosome population. Again, non-reduction would be expected to be facultative, but the reduced fertilized egg-cells could not be expected to maintain 54±-chromosome populations, because 54± is the triploid condition, therefore unbalanced, and in addition probably would be very non-fruitful.

Next consider plants of the genotype *AAbbCC*. The majority of the egg-cells

of such a plant would not be fertilized and hence these non-fertilized egg-cells would not produce progeny. The remaining small percentage of reduced eggs would be fertilized, and hence some progeny would be produced which could maintain this genotype in the population.

The *AABBcc* individual could survive very well in the population, since both reduction and fertilization would be occurring, and there would be no discrimination against *AABBcc* individuals other than the effect of recessives upon the ability of individuals which possess them to survive. It is a well known fact that most recessive mutations in the homozygous condition are at least somewhat detrimental to the development of the diploid individuals in which they occur.

This shows that all these recessive genes could be carried along to some extent in the double recessive condition as well as to a greater extent in the heterozygous condition in the diploid populations. Then the original 36+-chromosome population from which both the present 36+-chromosome populations and present apomictic polyploid populations evolved could very well have carried the recessive genes *a*, *b*, *c*. Eventually, by chance, hybrids could have been formed between two plants of the genetic constitution *AaBBcc* and *AaBbCC* (other genotypes would do equally well) to give an individual of the genetic constitution *aaBbCc*. Since the majority of the egg-cells of such a plant would be non-reduced, by further cross fertilization an individual or individuals of the genetic constitution *aaaBbbCcc* could arise. These individuals would be expected to have non-reduction of a large proportion of the egg-cells, failure of fertilization of a fair proportion of these non-reduced egg-cells, and, moreover, a number of these latter should be able to develop without fertilization. In other words, a number of plants, or a plant, with the genotype *aaaBbbCcc* would have arisen that reproduce to some extent by means of diplospory in which the semi-heterotypic division and restitution nuclei are occurring. The occurrence of the semi-heterotypic division would allow for segregation of genes without reduction of chromosome number, and hence some of these individuals produced by pseudogamous diplospory should be of the genetic constitution *aaabbbccc*. This plant or these plants would be facultative apomicts and have 54± chromosomes. Since these are facultative apomicts and the three major processes giving rise to apomixis are also facultative, it is apparent that 72±-chromosome apomicts could arise from 54±-chromosome apomicts by fertilization of a non-reduced egg-cell with an 18±-chromosome gamete.

Evidence that this does occur was obtained by the self-pollination of a 54±-chromosome hybrid involving a 36+-chromosome guayule and mariola (*Parthenium incanum* Gray), or a hybrid carrying a high proportion of the mariola gene complex. This hybrid under controlled self-pollination not only gave rise to a predominant number of 54±-chromosome apomictic progeny but produced several plants which had 72±-chromosomes.

Also, there is some indirect evidence that apomictic 54±-chromosome plants and 72±-chromosome plants are still arising from the 36+-chromosome plants. Collection 4257 arose from seed collected from an individual plant. The plants of this collection are facultative apomicts, and the great majority of them have 54± chromosomes. A few plants of this collection have 81±

chromosomes. A sister collection 4258 also arose from seed collected from an individual plant of this same population. The great majority of the plants of this collection had 72± chromosomes. Likewise, a few plants of this collection had 108± chromosomes. Both of these collections were made by DR. W. B. MCCALLUM and came from the same natural population as collection 4255 made by the author at the same time as these other two collections. This latter collection (4255) is a non-selected sample of seed taken at random from more than 100 plants of the natural population. More than 200 plants of this collection have been grown, and although cytological counts have been made of only a comparatively few, other studies such as size of pollen grains, percentage of aborted pollen grains, and morphological appearance of the plants have failed to reveal any plants having more than 36+ chromosomes. These two exceptional polyploid apomictic plants could well have arisen from the 36+-chromosome plants by some such method as previously outlined. They are difficult to explain as mixtures, since all of the near-by natural populations from which seed was collected had 36+-chromosome plants.

If the hypothesis presented to explain the origin of the polyploid apomictic collections is correct, the question arises as to why 36+-chromosome apomictic populations have not been found. This is a particularly pertinent question because the *aaBbCc* plants which, under the hypothesis formulated, occur in the 36+-chromosome population should (because non-reduction is facultative) give rise to some plants with the genotype *aabbcc*. Such plants would have 36+ chromosomes and would be facultative apomicts. There are a number of factors which operated against the establishment of pseudogamous diplosporous apomicts in diploids that either do not operate in polyploids or their detrimental effects are much less.

The first of these that will be considered is the frequency of occurrence of the diploids and polyploids from *aaBbCc* genotypic individuals. Since *aa* provides that a high proportion of the egg-cells of such an individual are non-reduced and *Bb* that a high proportion of these egg-cells are fertilized, most of the offspring would have 54± chromosomes (36+18). Then from the standpoint of number of individuals produced, the polyploid would have a decided advantage over the diploid.

Another factor is that recessive mutations in the homozygous condition are usually at least somewhat detrimental to the development of the individual possessing them and thus would materially affect that individual's ability to survive and become established under competition. This detrimental effect is usually much more pronounced in diploids than in polyploids. In this same connection, since these steps are facultative and show different degrees in frequency of expression, pseudogamous diplospory would be expected to be more frequent in the triploids of the genetic constitution *AaaBbbCcc* than it would in the diploids of the genetic constitution *AaBbCc*. In fact, many instances are known in quantitative inheritance in which two recessives are partially dominant to one of the ordinarily dominant genes. Here again the proportion of apomicts in the progeny would be higher for the polyploid. Another possibility is that perhaps neither the *aabbcc* genotype in the diploid nor the *aaabbbccc* genotype in the triploid could survive under competition when these three

pairs of genes first became combined into one individual, but that plants of the genotype *AaBbCc* and *AaaBbbCcc* could survive. In such an event it can be seen that the probability of apomixis becoming established would be better for the polyploid, since the chances would be greater for the development of a system of modifying genes favorable to the eventual survival of the triple recessive genotypes.

Still another factor of decided importance is incompatibility (see POWERS and ROLLINS 1945). It was found that the individual plants of the different chromosome groups gave the following mean percentages of seed which produced seedlings when they were self-pollinated and when pollination was not controlled: the 36+-chromosome group 0.6 ± 0.13 and 22 ± 0.42, and the $54 \pm$-chromosome group 4.3 ± 0.27 and 19 ± 0.41, respectively. Since a high degree of self-incompatibility is characteristic of the existing populations of 36-+ chromosome populations and since this same phenomenon to a lesser degree is shown by the $54 \pm$-chromosome populations, it follows that such probably was the case as regards the original 36+-chromosome plants from which the present sexual diploids and the apomictic polyploids were derived. Then, since the apomicts are pseudogamous, it is apparent that these self-incompatibility genes would greatly decrease the possibility of apomixis becoming established in diploids, because contemporary strains with different compatibility genes would be required for the establishment of populations largely reproducing by means of pseudogamous diplospory. Again, in the case of incompatibility genes the diploid as compared with the polyploid is discriminated against. Here it should be noted that incompatibility in the case of the $72 \pm$-chromosome group is not so important an item, since self-pollination produced, on the average, almost as great a percentage of seeds capable of producing offspring as did the non-controlled pollinations.

Then the factors disproportionately discriminating against the establishment of pseudogamous diplospory in diploids as compared to polyploids may be listed as follows: non-reduction followed by fertilization, when this process is facultative, provides that the larger proportion of the offspring be polyploids; it is more difficult for recessive mutations, due to their detrimental effects, to become established in diploids than it is for them to become established in polyploids; and finally, incompatibility genes make it necessary, in the case of diploids, that contemporary strains of pseudogamous apomicts become established before populations can exist which largely reproduce by means of pseudogamous diplospory.

These considerations emphasize the great difficulty that apomixis would have in becoming established in the diploid forms as compared with the polyploid forms. However, it would not be impossible and, therefore, the occasional diploid apomicts reported in agamic complexes would be expected.

On the basis of the hypothesis advanced to explain the evolution of apomixis and polyploidy, it would be equally difficult for sexuality to become established in the polyploids. Since the triploids are unbalanced types in respect to chromosome number, it is apparent that they could not be maintained by normal sexuality. The four genomes of the tetraploid ($72 \pm$-chromosome group) would be homologous, making it difficult for sexuality to become established in these

forms, due to the irregularities which usually occur during meiosis when such a situation exists.

It is evident that many plausible modifications of the hypothesis advanced could be made. It does not seem necessary to mention more than a few of them in the present paper, since the fundamental considerations are the same. One is that some of the mutations could have occurred in the polyploid form, such as the change from *B* to *b*. Another is that more gene pairs could be evolved in the differentiation of each step. Still another is that the 72±-chromosome apomicts could have become established as homogeneous populations before the 54±-chromosome group and that this latter group came about through hybridization between plants of the 72±-chromosome group and plants of the 36+-chromosome group. That the 72±-chromosome group may have become established as natural populations before the 54±-chromosome group is plausible since the first generation progeny of 42163 produced 72±-chromosome plants and 108±-chromosome plants, whereas the second generation progeny produced 72±-chromosome plants, 108±-chromosome plants, and 144±-chromosome plants, as well as plants with other numbers, showing that a series of chromosome number groups are produced in succeeding generations. Lastly, it should be mentioned that interspecific hybridization as well as intraspecific hybridization could have played some role and undoubtedly did in a number of agamic complexes.

Before closing the discussion it should be pointed out that the hypothesis advanced for the explanation of the evolution of the pseudogamous polyploid apomicts in Parthenium explains why there is an association between sexuality and diploidy, between polyploidy and apomixis, and in many agamic complexes between hybridization, polyploidy, and apomixis. Moreover, the hypothesis shows why both STEBBINS (1941) and FAGERLIND (1944) are correct in their assumptions. As STEBBINS concludes, polyploidy is conducive to the establishment and conservation of apomixis. Likewise, as FAGERLIND (1944) points out, apomixis in some families, genera, and species is conducive to the establishment and preservation of polyploidy. In fact, in Parthenium it seems highly probable that both apomixis and polyploidy evolved together and that either one would have had great difficulty in becoming established or surviving without the other.

SUMMARY

Ninety percent of the progeny of an individual plant growing on the 02 Ranch in Texas was aberrant. Further progeny tests including three generations and a large number of plants showed that this individual plant and the majority of its progeny were abnormal facultative amphimicts (non-reduction in chromosome number followed by fertilization being the predominant method of reproduction).

In addition to abnormal facultative amphimixis, pseudogamous diplospory and normal sexuality were found to be occurring in the material studied. It seems probable that non-segregating apomixis was occurring, also. All of these methods of reproduction probably occur in one plant.

All of these processes involving methods of reproduction are quantitative—

that is, one of degree. This is shown by the fact that they are not distinct, but grade into each other. For example, pseudogamous diplospory represents steps between normal sexuality and non-segregating apomixis.

The number of major gene pairs differentiating fertilization from failure of fertilization in this material was found by progreny tests to be comparatively few, probably not more than two. Fertilization was at least partially dominant to failure of fertilization.

A hypothesis as to the evolution of apomixis and polyploidy in agamic complexes is developed and discussed. It seems likely that apomixis and polyploidy had a similar origin in other genera and species.

At least three major steps are involved in the evolution of apomixis from normal sexuality—namely, (1) failure of reduction in number of chromosomes, (2) failure of fertilization, and (3) development of non-reduced egg-cells without them being fertilized.

That it would be vastly more difficult for apomixis to become established in diploids than in polyploids is shown. Likewise, it is shown that in these agamic complexes such as is present in Parthenium, normal sexuality would have difficulty in becoming established in the polyploids.

Hybridization must have played an important part in the evolution of apomixis and polyploidy; probably both intraspecific and interspecific hybridization were involved.

In Parthenium, the evidence is convincing that both apomixis and polyploidy evolved together, and either one would have had great difficulty in becoming established and surviving without the other.

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A SURVEY OF CYTOGENETIC CAUSES OF UNFRUITFULNESS IN THE TOMATO

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UNFRUITFUL plants are a familiar sight in fields of the heavily fruiting tomato varieties late in the harvest season. Such plants are rendered conspicuous by their large moundlike vegetative growth, in contrast to the much lower and smaller growth of normal plants (fig. 1). Colloquially they are known as "bull," "buck," or "male" plants. The tendency to ascribe maleness to them is understandable. Considering the causes of the barren condition, however, these names are inappropriate: not one unfruitful plant of 66 studied in the present survey exhibits greater male than female fertility in breeding tests; moreover, in certain plants the direct antithesis to a "male" plant is found in complete pollen sterility and normal ovule fertility.

The unfruitful tomato plant owes its unique appearance to its very different physiological condition. It diverts into vegetative growth the food reserves which in normal plants are used to produce fruits. With very few or no fruits to support, it grows two or more times as tall as the fruitful plant. The total amount of vegetative growth of an unfruitful plant usually greatly exceeds that of a normal one (fig. 1). Flowering of such plants continues indefinitely, whereas in normal plants a heavy setting of fruits often terminates flower production. MURNEEK (1926) observed similar features in tomato plants from which fruit had been removed. He also discovered that developing fruits divert much of the nitrogen from the vegetative parts, and thereby explained the close correlation between vegetative growth and absence of fruit. Unfruitful plants develop a more extensive root system, which may account for their greater ability to withstand drought. Moderate frosts that may kill all surrounding plants often cause little or no damage to unfruitful ones. In some unfruitful plants this frost resistance appears to be an inherent property of the foliage; in others, it is vested in the dense, bushy habit of the unfruitful type: the center remains undamaged, thanks to the cover provided by the outer shoots, which, even if injured, are less noticeable than the healthy green center. Differing, therefore, in these various respects, the unfruitful plant is conspicuous in the field even at a great distance.

The great diversity of leaf shapes and the variations of other plant parts observed in unfruitful tomato plants suggest that the causes of unfruitfulness may be equally varied. According to LESLEY and LESLEY (1939) and RICK (1944), genetic male sterility may account for unfruitfulness in certain plants. J. W. LESLEY (1928) reported the discovery of several triploids that undoubtedly were very sterile. In a subsequent publication (LESLEY 1929) he described two tomato plants which evidently owed their sterility to an epidermal layer of mutated cells. CURRENCE (1944) found a mutant tomato possessing a style

exserted 2 to 5 mm beyond the summit of the anther column; by virtue of this long style, natural pollination is hindered, and fruitfulness is consequently impaired. In many of the tomato varieties popular in England—for instance, Balch's Fillbasket and Plumpton King—diploid plants appear which produce their first inflorescence after the tenth or higher node, not in the normal position between the sixth and ninth nodes, and which to varying degrees produce less fruit than normal plants (HUDSON 1939). HUSKINS and CRANE (1930) at first attributed the infertility of these "rogues" to a wide constriction in one pair of chromosomes. More recently CRANE (1939), on the basis of very unstable transmission rates, has proposed a highly mutable gene to account for this character.

Numerous diseases are known to reduce the fertility of the tomato. In the writer's observation, plants infected with cucumber mosaic are often unfruitful; but when plants are diseased to the extent that they fail to fruit, they are also so stunted vegetatively that they do not exceed the growth of normal fruitful plants. Diseased plants differ in other respects from healthy unfruitful plants and were therefore not included in this study.

Being so conspicuous, unfruitful tomato plants can be obtained in large numbers by scanning extensive plantings during the late harvest season, even though unfruitfulness apparently does not occur in more than 0.12 per cent of all plants. Appearing so rarely, these plants have relatively no effect upon total yield; hence the growers seldom remove them from the field.

In the present survey 66 unfruitful plants were secured by scanning six plantings in the lower Sacramento Valley. The study was limited to three canning varieties of this district: Early Santa Clara, characterized by indeterminate plant growth and very large fruits of 15 to 25 locules; Pearson, a determinate variety that produces fruits of average size having five to seven locules; and San Marzano, resembling the more familiar King Humbert—an indeterminate type producing elongate, irregularly pear-shaped fruits, typically of two locules. Early Santa Clara and San Marzano are very distinct and apparently unrelated, but Pearson was partly derived from a strain of Santa Clara. They were selected because of their differences to represent a wide range of tomato varieties. Plants of intermediate unfruitfulness were rarely encountered; when found, however, they were included among the samples collected. The conditions responsible for unfruitfulness of the plants studied here alter gametogenesis profoundly. For this reason, fortunately, difficulties of dealing with unfruitfulness quantitatively are avoided.

In order to estimate the frequency of unfruitful plants in these three varieties, only those fields were scanned that had not previously been rogued for unfruitfulness. Despite this precaution, the obtained estimates are probably deficient because haploids and certain other unfruitful types produce weak seedlings, which no doubt suffer discrimination at the time of transplanting seedlings to the field. Every unfruitful plant encountered was collected so that the samples would exemplify the different types of sterility and their relative proportions.

Cuttings of each unfruitful plant were rooted, and plants thus obtained were grown in the greenhouse during the winter; another generation grown the following summer was propagated in the same manner. Analysis of one unfruitful plant of Pearson was not completed because the determinate habit of this variety precludes or greatly limits shoot growth of cuttings. Any seeds produced were collected and sown whenever seedlings could be used to advantage.

Root tips were taken from rooted cuttings, fixed in Karpechenko's fluid, imbedded, sectioned, and stained in iodine-gentian violet for studies of chromosome number. Counts thus made were verified by duplicate collections and were reliable except in the frequently encountered cells of doubled chromosome number. Some root tips were sectorial chimeras of cells having the somatic number and those having twice the somatic number; others consisted wholly of cells of the doubled number. This phenomenon has been observed in tomatoes by MARGARET M. LESLEY (1925) and in many other plant species by other workers. Figures in root tips from one of the haploid plants, for example, were all diploid and consequently very misleading.

This somatic doubling in root-tip tissue demanded checking in shoot cells. Smears of PMC's were examined in certain plants; but this method could not be used generally because flower buds of some plants were not available and premeiotic disintegration of sporogenous tissue occurred in others. Smears of shoot meristems and embryonic leaves, adopted as an alternative method, proved satisfactory for confirmatory counts. Several shoot growing points collected from each unfruitful plant were fixed in Carnoy's acetic-alcohol. After fixation, these were subjected to hydrolysis and smearing as outlined by BALDWIN (1939). One or two smears from each plant provided sufficient mitotic metaphases to permit consistent determination of diploidy or euploid variations, although the figures were usually not clear enough to permit counting the exact number of chromosomes (fig. 11, 12, 13). The opportunity to make counts within two to three hours of collecting is another advantage of this method. No chromosome doubling whatever was found in these shoot meristems, in contrast to the behavior in root tips of the same plants. RUTTLE (1928), who observed copious doubling of chromosome number in roots of haploid *Nicotiana Tabacum*, also noted the absence of doubling in shoots (smears of PMC meiosis).

Where female gametophytes were studied, ovaries were fixed in FAA (formula given by COOPER 1931) and stained in Heidenhain's haematoxylin according to the schedule published by ESAU (1944). Genetic data were obtained from a winter generation in the greenhouse and from a second generation grown in the field the following summer. Most controlled pollinations were made in the greenhouse.

By application of the foregoing techniques, unfruitfulness was largely resolved to gametic sterility, which in all investigated plants had a cytogenetic basis.

CHROMOSOME NUMBERS OF UNFRUITFUL PLANTS

Table 1 shows the relative abundance of all unfruitful plants and the chromosome numbers of the unfruitful plants of each of the three varieties studied. Comparisons of the varieties reveal a striking similarity in types and frequencies of chromosomal variants. Triploid plants comprise nearly two thirds of all unfruitful plants obtained, the other numbers found being, in order of abundance, diploid, tetraploid, haploid, and trisomic. The appearance of these chromosomal types in nearly the same proportions in these three relatively dissimilar varieties suggests that this composition is typical of most tomato varieties and perhaps of the species as a whole.

TABLE 1

Chromosome numbers of a random sample of 66 unfruitful plants in three tomato varieties.

VARIETY	NUMBER OF PLANTS HAVING THE CHROMOSOME NUMBERS:					TOTAL	PERCENTAGE OF ALL PLANTS IN FIELD
	12	24	25	36	48		
Early Santa Clara	2	9	1	29	1	42	0.12
Pearson		3		9	1	13	0.11
San Marzano		2	1	7	1	11	0.12
Total	2	14	2	45	3	66	

Notable agreement between varieties is also observed in the frequency of all types of unfruitful plants—namely, about one per 1,000 fruitful plants. According to less detailed observations by the writer in other varieties, and the following observations by others, this frequency of unfruitful plants is typical of American tomatoes. Several organizations that grow large acreages were polled in order to ascertain the abundance of sterile plants in other varieties. Information was kindly furnished by E. L. ABERNETHY of the Haven Seed Co., R. H. COULTER of the Ferry-Morse Seed Co., D. M. GLICK of Glick's Seed Farms, C. E. MYERS of the Department of Horticulture at the Pennsylvania State College, C. P. SCHAFFER of the Francis C. Stokes Co., and M. ZOBEL of the Dessert Seed Co. According to these observers, the frequency of unfruitful plants determined in this study prevails for at least eight varieties other than the three investigated here.

Exceptionally high frequencies were reported in a single season in Earliana and Rutgers, varieties ordinarily characterized by about one unfruitful plant per 1,000. Although the chromosome numbers of these abnormally plentiful plants were not determined, the growers' descriptions suggest that they were mostly polyploids. The reason for such fluctuations is obscure. As discussed later, it might, however, be related to seasonal influences during gametogenesis, fertilization, and early development of the embryos of the seed used in these plantings.

Triploids, the most abundant of all unfruitful types in the present survey, are less common (three in 9,000) in the variety Dwarf Aristocrat (LESLEY and LESLEY 1930).

NATURE OF STERILITY OF UNFRUITFUL HETEROPLOIDS

High seed sterility and attendant unfruitfulness have been observed previously in triploid tomatoes by J. W. LESLEY (1928) and in haploid tomatoes by LINDSTROM (1929). SANSOME (1933), LESLEY and LESLEY (1930), and LINDSTROM and HUMPHREY (1933) report considerable seed sterility in tetraploid tomatoes, although not so high as in the tetraploids considered here. These euploid variants are all characterized by high abortion, both of pollen and of embryo sacs (table 2). Tetraploids are the most fruitful of the three types, although the number of fruits produced is only a small fraction of the yield of fruitful diploids. The tetraploids also have the highest mean number of seeds. Their relatively higher fertility is correlated with a higher gametic fertility, which, in turn, is conditioned by a higher proportion of balanced chromosome complements contributed to the gametes than that of the haploids and triploids. The number of fruits produced by haploids and triploids varies from none to 20 depending upon variety and size of plant; a mean number of one seed is produced per fruit. Meiosis was studied in PMC's of the two haploid plants and in a few of the triploids. In these types all but a very small fraction of the spores receive an unbalanced chromosome complement, a fact accounting for the very large percentage of aborted gametes. The course of meiosis resembled that described for haploid tomatoes by HUMPHREY (1934) and NEWCOMBER (1941) and for triploids by J. W. LESLEY (1928) and therefore need not be elaborated here. The pollen samples of haploid plant No. 16-64 revealed no normal grains whatever, yet this plant produced 15 fruits containing a total of 14 seeds when growing in the field surrounded by fruitful diploids. All its progeny are diploid, unreduced eggs of the haploid being fertilized by haploid sperms of normal pollen tubes. Very likely, therefore, natural cross-pollination by the surrounding diploid plants accounts for the fruits and seeds produced by this haploid and probably also by the triploids and the other haploid. The tetraploid number found in the progeny of two of the tetraploids points to selfing as the likely mode of pollination involved in their production of seed and fruit in the field.

Of the two trisomics discovered, one was lost because cuttings failed to grow. The other, No. 16-41 was found in the variety San Marzano. It is a dwarf plant resembling tetraploids of this variety, for which it was at first mistaken. Ovular sterility as determined by applying viable pollen on stigmas of this plant is almost complete despite the normal appearance of 57 per cent of the ES's (table 2). Trisomics are not generally so barren as this; possibly, therefore, the scanning method employed here reveals only the most sterile trisomic types and does not give an accurate estimate of the frequency with which trisomics naturally occur.

The abundant literature concerning the discovery of all types of heteroploidy in members of twin and multiple seedlings led the writer to test polyembryony as a source of these tomato heteroploids. Seeds of ten plants were collected and sown separately, and another lot of seed was massed from a larger number of plants, of the variety Early Santa Clara. These seeds were sown on

TABLE 2

Proportion of sterile gametes in heteroploid, normal diploid, and mutant diploid plants.

PLANT NO.	VARIETY	CHROMOSOME NUMBER	BREEDING FERTILITY*		MEIOSIS IN PMC	NUMBER POLLEN GRAINS COUNTED	PERCENTAGE ABORTED POLLEN†	NUMBER EMBRYO SACS COUNTED	PERCENTAGE ABORTED EMBRYO SACS‡
			POLLEN	OVULE					
145-1	E.S.C.	24	Fertile	Fertile	Normal	558	12	155	12
132-1	S.M.	24	Fertile	Fertile	Normal	502	7	199	3
16-58	E.S.C.	12	Completely sterile	Very sterile	All univalents	230	98		
16-64	E.S.C.	12	Completely sterile	Very sterile	All univalents	216	100		
16-41	S.M.	25		Very sterile		94	19	141	43
16-46	E.S.C.	36	Very sterile	Very sterile	Mostly trivalents	218	90	151	92
16-40	S.M.	36	Very sterile	Very sterile		230	93	139	83
16-43	S.M.	48	Very sterile	Very sterile		203	93	179	57
117-1	Pearson	48	Very sterile	Very sterile		223	86	238	77
16-5	E.S.C.	24	Partly fertile	Fertile	Normal	212	19	194	14
16-9	E.S.C.	24	Very sterile	Very sterile	Normal	236	10	162	87
16-25	Pearson	24		Very sterile		216	23		
16-31	Pearson	24	Completely sterile	Fertile	Normal	None produced		104	3
16-44	S.M.	24	Completely sterile	Partly fertile	Does not occur	None produced		181	69
16-63	E.S.C.	24	Completely sterile	Fertile	Normal	226	100	129	23
16-3	E.S.C.	24	Completely sterile	Completely sterile	Normal	227	45	166	41
16-4	E.S.C.	24	Completely sterile	Completely sterile	Normal	236	84	249	100
16-29	E.S.C.	24	Completely sterile	Completely sterile	Normal	547	97	342	100
16-38	S.M.	24	Completely sterile	Completely sterile	Asynaptic	237	94	201	99
16-56	E.S.C.	24	Completely sterile	Completely sterile	Normal	217	79	273	100

* Breeding fertility: degree of fertility as determined by use of pollen and ovules of given plant in breeding tests.

† Aborted pollen: proportion failing to stain in aceto-carmine; mean of 2 or 3 separate collections.

‡ Aborted embryo sacs: proportion of collapsed or otherwise abnormal embryo sacs in ovaries sectioned at anthesis; mean of percentages in 3 to 5 ovaries.

PLATE I

FIGURE 1.—Unfruitful triploid plant in field of the variety Pearson.
FIGURE 2.—(a) Fruit with indehiscent style of mutant No. 16-5. (b) Normal fruit.
FIGURE 3.—Fruit and inflorescence of proliferated mutant.
FIGURE 4.—(a–c) Flowers of apetalous mutant No. 16-9. (d) Normal flower of same variety.
FIGURE 5.—(a) Flower of male-sterile and (b) flower of male-fertile segregant in F_2 of No. 16-44.

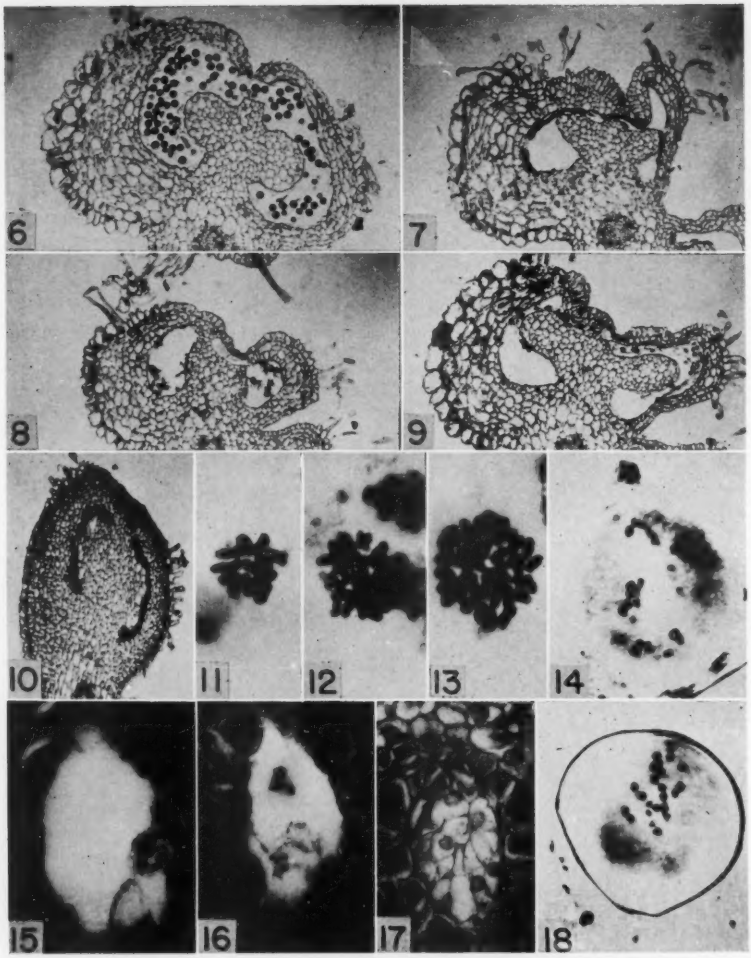

PLATE II

FIGURES 6 TO 10.—Cross-sections of anther locules (×75). Figure 6.—Male fertile anther. Figure 7.—Anther of male-sterile No. 16-31. Figure 8.—Anther of male-sterile No. 16-44. Figure 9.—Anther of male-sterile No. 16-63. Figure 10.—Anther of male-sterile No. 42L1-1.

FIGURES 11 TO 13.—Metaphases of somatic mitoses in smears of shoot meristems (×1250). Figure 11.—No. 16-58 (haploid). Figure 12.—Diploid. Figure 13.—No. 16-49 (triploid).

FIGURES 14 AND 18.—Smears of MI in PMC's of No. 16-38 (asynaptic diploid) (×850). Figure 14.—Anaphase showing two bivalents. Figure 18.—Metaphase.

FIGURES 15 TO 17.—Longitudinal sections through ovules (×400). Figure 15.—Normal embryo sac of No. 16-43. Figure 16.—Collapsed embryo sac of No. 16-44. Figure 17.—Cavity of embryo sac filled with undifferentiated cells of No. 16-29.

moistened blotting paper. As they germinated, they were searched for multiple seedlings. Conditions were favorable, as attested by the fact that germination in all lots ranged between 96.1 and 99.7 per cent. Weaker members of multiple seedlings are not likely to emerge unless optimal conditions for germination are maintained as found in Linum (KAPPERT 1933) and in *Asparagus* (RICK and RANDALL unpublished).

Four pairs of twin seedlings, found in a total of 18,672 germinated seeds, were fixed, imbedded, and sectioned. Determinations based on metaphase figures and nuclear size indicated seven of the seedlings to be diploid and one to be haploid. Polyembryony is thus inadequate to account for the heteroploids described here, for even if heteroploids of all kinds were discovered as often in twin seedlings as the haploids found here, their frequency, 0.005 per cent is significantly much lower than the 0.095 per cent discovered in the field. A test for significance of difference between very low percentages, based on binomial expansions, has been devised by STEVENS (1942), who revealed the fallacy of relying upon $\sqrt{p(1-p)/n}$ as the standard error of such low percentages. The fiducial limits at the one per cent level as calculated by STEVENS' method do not overlap, the upper limit for heteroploids in twin seedlings being 0.040 per cent and the lower limit for the frequency of heteroploids in the field being 0.065 per cent. As a source of haploids alone, polyembryony could account for the 0.004 per cent observed in the field.

This test does not exclude the possibility that heteroploids might start development as multiple embryos. Members of the overpopulated ovule may die before the stage of germination, as has been demonstrated in Citrus by FROST (1926). The fact that heteroploids might originate in this manner would not be revealed by mere examination of germinated seed.

Although the functioning of non-reduced parental gametes may account for the tetraploid plants, it must explain the appearance of the triploids. The tetraploids could also arise from somatic doubling either of the zygote in the immediate generation or of later developed tissue or zygotes in preceding generations. No 2n:4n chimeras were found, hence the doubling did not occur in post-zygotic development of the discovered plants. That high temperature may be responsible for the production of diploid gametes is suggested by the non-reduction during hot weather observed in tomatoes by LESLEY and LESLEY (1943). High temperature shocks will likewise induce somatic polyploidy (RANDOLPH 1932, and others). An environmental effect is also suggested by a fact already mentioned—namely, that polyploid plants may be unusually numerous during certain seasons in varieties which in other years show a typically low frequency. A genetic effect is probably not involved, because it might reasonably be expected to be maintained for several years in view of the methods of seed production currently employed.

The relatively rare trisomic plants found could originate from chromosomal nondisjunction at meiosis of the parent plant or from seed produced by triploids. Although triploids set very little seed, they are the commonest of all unfruitful types, occurring at the rate of one per 1,200± plants.

NATURE OF STERILITY OF THE UNFRUITFUL DIPLOIDS

Of the 14 unfruitful diploids, 11 were subjected to comparable cytogenetic tests. Collections were incomplete from the remaining three, the clones having been lost during the study.

Prominent gross morphological abnormalities are exhibited by three of the diploids. The rare coincidence of aberrant morphology and unfruitfulness appearing simultaneously in the same plant strongly suggests that one gene determines both characters or that a small chromosomal segment including a different gene for each effect is deficient. Association of sterility with morphological change without exception has not yet been demonstrated by breeding tests in each case.

One plant, No. 16-5, characteristically produces smaller fruits than corresponding fertile plants; and its fruits develop a pronounced beak at the stylar end (fig. 2). Styles do not dehisce after flowering, but persist on the fruit. Although the distal end of the style may wither, the basal portion continues to enlarge to form part of the pointed end. This mutant may be identical with the "beaked-fruit" type described by LINDSTROM (1932, 1941). Despite the normal appearance of a considerable proportion of the gametes, this form sets only occasional fruits. Reciprocal crosses with normal diploids indicate a much greater defectiveness of pollen than of ovules. Semisterility of pollen therefore accounts for the unfruitfulness of this plant. The indehiscent style and the unfruitfulness are reproduced uniformly in the progeny secured from self-pollination. Transmission to the progeny leaves no doubt that this type is genetically determined, but no breeding record is yet available to indicate the nature of the inheritance involved.

Another gross morphological mutant, No. 16-9, is a normal plant in every respect except that the corolla is greatly reduced and that ovule and pollen sterility are high (table 2). The degree of development of petals (fig. 4) is variable, but in no case do any number of the petals reach normal length. Despite repeated attempts, no seeds have been produced by self-pollination. The few F1 seedlings secured from crosses with normal plants were normal in fertility and in structure of corolla. Although a second generation has not yet been grown, this character, like the majority of the known mutant characters of the tomato, is probably conditioned by a recessive gene.

The third mutant of this group, No. 16-25, was discovered as a sport on an otherwise normal plant. Half the plant was characterized by high pollen abortion, extreme unfruitfulness, and absence of the larger trichomes on stems and petioles, which rendered it glabrous to the naked eye. These attributes did not appear in the first generation of 26 plants and a second generation of 81 plants. In all respects this mutant resembles the two described by J. W. LESLEY (1929) and explained as chimeras in which only the epidermal layer was composed of mutant cells.

Another morphologically aberrant plant, discovered in 1941 as an unfruitful plant by G. C. HANNA, although not found in this sample of 66 plants, is

worthy of mention here. Illustrated in figure 3, its flowers are enlarged in the center by a great proliferation of abortive anthers and filamentous petals. The fruit, which is developed only after the application of normal pollen to the stigma, is very irregularly shaped; and the brushlike proliferation of flower parts persists at its stylar end. Since all attempts failed to induce seed formation, no data concerning the inheritance of this character could be obtained.

The unfruitfulness of three other diploid plants—one in each of the three varieties—is caused by genetic male sterility. In gross morphology, each is identical with fruitful plants of the corresponding variety except for lighter color, various degrees of shrunkenness of the anthers, and differences in growth incurred by unfruitfulness, the first two differences being related to the disturbance of normal pollen production. Although no viable pollen is yielded by any of these mutants, each set a few fruits in the field—the products of outcrossing

TABLE 3

Segregation of male sterility in F_2 generations of three mutants.

GRAND-PARENTAL PLANT NO.	VARIETY	F_2 PROGENY NO.	OBSERVED NUMBER OF PLANTS		EXPECTED NUMBER OF PLANTS
			MALE FERTILE	MALE STERILE	
16–31	Pearson	136	21	9	22.5:7.5
		139	40	12	39:13
16–44	San Marzano	134	21	8	21.75:7.25
		138	4	2	4.5:1.5
16–63	Early Santa Clara	142	5	2	5.25:1.75

to normal male-fertile plants as determined by subsequent breeding tests. The production of fruits and seeds by male-sterile plants thereby gives, for tomatoes, some measure of the amount of natural cross-pollination, which, though small, occurred in each of the three varieties. Plants from the hybrid seed thus secured were male-fertile in every case, a fact indicating the recessiveness of these characters. The typically monogenic ratios obtained in F_2's (table 3) delineate the nature of inheritance. The F_2 culture derived from No. 16-63 is obviously not large enough to disprove significantly other types of inheritance; yet the conformity of segregation to simple Mendelian inheritance, resembling the other male-sterile mutants in this study and literally scores of male-sterile types in other species reported elsewhere leaves little doubt concerning the nature of its genetic determination. The classification in table 3 is based on examination of anther smears, although male-sterile individuals of each mutant can be distinguished readily from their male-fertile sibs by the appearance of their anthers.

In all the foregoing respects the three male-sterile mutants resemble the one previously described by the writer (1944); nevertheless, no two of the four are alike in the nature of development of PMC's or male gametophytes. The differences in anther development hint that different gene mutations are involved, although, admittedly, the markedly different genotypes of the three varieties might modify phenotypic expression of a single gene to a similar extent. Tests of allelism are now in progress. Unless these prove otherwise, the genes are assumed to be at different loci, a different *ms* symbol being tentatively assigned to each mutant.

Development of sporogenous tissue and male gametophytes of the four mutants proceeds as follows:

1. No. 42Li-1 (*ms1*) (Rick 1944). No pollen in any condition is present in mature anthers. Anther walls remain shrunken because the locules fail to enlarge (fig. 10). PMC's are differentiated and appear normal until the earliest stages of meiosis, but most have collapsed by midprophase of meiosis. In all smears the latest developmental stage found was a typical diakinesis figure in one PMC.

2. No. 16-31 (*ms2*) Pearson. Smears of mature anthers reveal no pollen, either normal or aborted. Sections (fig. 7) show concave anther walls and a small amount of degenerated material present in the locule. Meiosis proceeds normally. Microspores degenerate shortly after formation of tetrads.

3. No. 16-44 (*ms3*) San Marzano. Flowers of the genotypes *Ms3ms3* and *ms3ms3* are compared in figure 5 to show the slightly more contracted and paler-colored anthers of the male-sterile flowers. Development of the sporogenous tissue is largely impaired in pre-meiotic development of PMC's. Although a few figures are seen in metaphase and telophase of the first meiotic division, no tetrads are encountered. Unlike *ms1*, collapsed cells are found consistently in anthers smeared at anthesis. These cells, which might be mistaken for aborted microspores, prove to be the remains of old PMC's (fig. 8).

4. No. 16-63 (*ms4*) Early Santa Clara. In smears of mature anthers a few aborted pollen grains are found (fig. 9). The time of breakdown in development is less precise than in the other mutants. Meiosis of most PMC's progresses normally, though division of many cells is delayed. Thus, while most material in a preparation has reached the tetrad stage, other PMC's are just entering prophase of the first division, and occasional PMC's degenerate before completion of meiosis.

The male-sterile type described by Lesley and Lesley (1939) differs from these four types in several respects. Anthers of the former are practically indistinguishable externally from fertile anthers; meiosis in PMC's is normal, and development of the pollen is arrested just short of maturity; and at least two recessive genes are necessary for its occurrence.

The discovery of these male-sterile mutants in each of the three varieties demonstrates that genetic male sterility can be obtained in a line of tomatoes by applying the scanning method and the cytogenetic tests used here, provided

sufficient plants are inspected. First generation hybrids between certain tomato varieties yield very heavily and are potentially of great practical value. The use of male-sterile mutants in the production of hybrid seed was proposed by BARRONS and LUCAS (1942). The advantages of using male-sterile mutants as female parents for this purpose are: emasculation is unnecessary; no danger of contamination by selfing exists; they are easily identified in the field; and since they depend on simple recessive genes, they constitute 50 per cent of the backcross progeny and therefore are easily obtained in quantity. Now, not only are the appropriate mutants available, but one can secure male sterility in any desired line or variety much more quickly by applying these techniques instead of the traditional method of backcrossing.

The five remaining diploid plants are completely sterile. Direct and reciprocal crosses with fertile diploids failed to induce the formation of fruits and seed in the relatively cool, humid conditions of the greenhouse in winter or in the warm dry environment of field culture in summer. These plants appear normal except for their unfruitfulness and have maintained their characteristics for two successive clonal generations. If their extreme sterility were caused by virus disease, one might anticipate changes in the foliage, stunting of growth, similarity of type of gametic sterility, and transmission of the sterility to nearby plants. Since these effects were not in evidence, virus disease cannot be considered a likely cause of the observed unfruitfulness. Breakdown of development of male gametophytes was found to be similar to the genetically determined types already described; but PMC's of one plant exhibit asynaptic meiosis, typical of the genetically determined asynapsis described in the literature. Probably, therefore, these plants, like the foregoing diploids, are genetic mutants; their genetic determination cannot be proved, however, since they did not arise in pedigree cultures and since their complete sterility prevents further breeding.

As in all the preceding examples, including the heteroploid variants, gametic sterility is largely responsible for the unfruitfulness of these plants (table 2). This situation was predicted by the aforementioned experience in attempted crosses.

Although sterility as recorded in table 2 is not always complete, notably in No. 16-3, the recorded percentages of aborted gametes apparently represent a minimum estimate. In examining pollen, for example, all grains that have contents stainable with aceto-carmine are scored as fertile. There can be no mistake in assuming that an unstained and usually collapsed grain is inviable; but the ability of a stainable grain to function in pollination (or of a plump seed to germinate) is not always certain. Other workers have experienced similar difficulty in identifying viable pollen.

Likewise, there is little doubt concerning the accuracy of scoring functionless embryo sacs; but the recognizing of certain embryo sacs as potentially viable can be questioned. Despite this uncertainty, fair agreement exists between the estimated proportion of sterile ES's and the degree of sterility as repre-

sented by the numbers of seeds produced. Thus the male-sterile No. 16-44 (*ms3*), although capable of setting fruits when crossed with normal pollen, produced a mean of only 10.5 seeds per fruit, whereas No. 134-4, a male-sterile granddaughter of this plant, averaged 25.1 seeds per fruit, and male-fertile sibs produced about 40 seeds per fruit. The proportions of fertile ES's of these plants, in corresponding order, are 31, 57, and 97 per cent. These two estimates of fertility thus agree in order of size. Though the two are not perfectly correlated, the degree of seed fertility is approximately predicted by the proportion of fertile ES's as estimated in fixed material.

An ovule was scored as fertile if it contained the typically distended, highly vacuolate ES with fusion nucleus and egg apparatus (fig. 15). Collapse of the ES may occur at various stages of maturation; it is indicated in the ovule at anthesis by the degenerate remains of the gametophyte in a smaller cavity, usually encroached upon by cells of the surrounding integument (fig. 16). Another type of sterile ovule (fig. 17) contains, not a mature ES, but a mass of undifferentiated cells more vacuolate than adjacent cells of the integument, and is also surrounded by radially elongated cells of the integument. The development of these aberrant structures will be described in a later publication.

The development of male gametophytes in these five completely sterile plants resembles that of the male-sterile plants, impairment occurring mostly in later stages of maturation.

In this respect plant No. 16-38 of this group deserves mention because the failure of its pollen production can be attributed to asynaptic meiosis. Pollen in mature anthers is highly aborted, the six per cent of grains with stainable cytoplasm being undersized and otherwise of doubtful viability. Microspores in all stages of development are extremely variable in size—a condition which suggested that apportionment of chromosomes to the spores, as in triploids, might be variable, and which led to the examination of meiosis. Paired chromosomes are occasionally seen at metaphase of the first meiotic division of PMC's of this plant (fig. 14). Typically, however, the chromosomes are all represented as univalents and are spread at random in the general equatorial region (fig. 18). Some figures in diakinesis exhibit bivalents, in which the pairing evidently denotes previously occurring chiasmata. In these respects the present example resembles the classical case of asynapsis in maize (BEADLE 1930). No offspring were obtained from this plant, despite the pollination of more than 100 of its flowers with normal pollen and despite as many attempts at reciprocal crosses.

Fertility studies were incomplete for the three diploid plants lost during the course of this study. None of the three produced normal pollen, and one in which ovule fertility was studied showed 35 per cent aborted ovules. Meiosis was found to be normal in PMC's of these three plants. According to all observations, any of these plants could be placed in one of the categories already discussed.

Of all the types of diploid unfruitful plants encountered in this survey, none

is comparable with the "rogue" described by HUDSON (1939) and analyzed cytogenetically by HUSKINS and CRANE (1930) and CRANE (1939). The rogue's characteristic dwarfness, delayed flowering, and varying degrees of fruitfulness approaching normal fertility did not appear in any plant here; nor did the present unfruitful types occur nearly so often as the rogues, which may constitute as high as 14 per cent of certain English varieties. The rogue is apparently absent from most American tomatoes. On the other hand, the cytogenetic types of unfruitfulness described here are suspected to occur in English varieties in addition to the more frequent rogues. JØRGENSEN (1928) described a triploid tomato secured from M. B. CRANE in the variety Balch's Fillbasket; however, he does not discuss its mode of origin.

ORIGIN OF THE UNFRUITFUL DIPLOIDS

The unfruitful tomato diploids are probably derived from gene mutations (whole gene deficiencies or intragenic changes). Although this source is strictly proved for only three of the 11 plants investigated, it appears likely to hold for the remainder, for reasons already discussed. Assuming these 11 plants to be homozygous for recessive mutations, a provisional estimate may be made of the rate of mutations affecting fruitfulness in the tomato.

Although certain features of the breeding mechanism of the tomato populations inspected are not completely understood, sufficient facts are known to permit the following estimate. Investigations have repeatedly demonstrated a very low percentage of natural cross-pollination in tomatoes. This relatively strict self-pollination provides the greatest opportunity for recessive gene mutants to be fixed in the homozygous condition. Seed for these commercial plantings is generally obtained from large numbers of plants, which, in turn, are derived from large populations from year to year. Any selection practiced is not likely to affect the numbers of unfruitful mutants observed. The relative reproductive rate of different plants within these populations is unknown; if great variations exist, any estimate of mutation frequency assuming equal reproduction rates would be seriously in error. Although slight variations of this kind probably exist, no marked differences have been observed by the writer; moreover, plants heterozygous for mutations affecting fertility were found to be as reproductive as non-mutant plants.

Assuming that these tomato populations consist of a great number of plants reproducing at equal rates and breeding in equilibrium, and that they reproduce exclusively by self-pollination, a plant heterozygous for the gene, *a*, conditioning sterility, will yield, in the next generation, $\frac{1}{4}AA:\frac{1}{2}Aa:\frac{1}{4}aa$. The recessive homozygotes, *aa*, (the unfruitful plants observed) produce no offspring or so few as to be negligible. The heterozygotes will in turn give rise to $\frac{1}{4}$ *aa* in the next generation, the numbers of unfruitful homozygotes thus being reduced by one half in each generation. If one new mutant is assumed to appear in each generation, the relative frequency of homozygotes in a given population may be represented as follows:

Generation	Total recessive homozygotes			
1				*Aa*
				↓
2			*Bb*	1/4*aa*
			↓	↓
3		*Cc*	1/4*bb*	1/8*aa*
		↓	↓	↓
4	*Dd*	1/4*cc*	1/8*bb*	1/16*aa*
	↓	↓	↓	↓
5	*Ee* 1/4*dd*	1/8*cc*	1/16*bb*	1/32*aa*
n	1/4 + 1/8 + 1/16 + 1/32 + · · · + $1/2^n \rightarrow 1/2$			

Thus on the average two new mutant heterozygotes appear in each generation for each mutant homozygote found in the field. Since two gametes fuse to produce each zygote, the gametic mutation rate equals the proportion of mutant homozygotes observed. This calculation does not take into account mutations occurring somatically. If each somatic mutation is taken to represent a mutant gamete produced by the preceding generation, the estimate hereby obtained will be deficient to an extent depending on the stage of development at which the somatic mutation occurred. Applying this calculation to the data obtained, the 11 mutants counted in approximately 55,000 plants represent a gametic mutation rate of about 0.02 per cent.

Since the only mutations counted in this survey were those adversely affecting fruiting, the estimate given above is obviously lower than the total rate of mutations having a gross morphological effect in this species. Thus mutations affecting such characters as fruit shape and color, stem color, and presence of trichomes would probably be overlooked, but the known mutants that markedly modify the habit aspect of the tomato plant—dwarf habit, potato leaf, determinate growth, wilty leaf, and wiry leaf—would certainly be discovered by the trained eye in the scanning method employed in this study. Thus plants of a differing variety occasionally misplanted in the fields were easily recognized at a distance even though they differed less in appearance than these mutants.

Another respect in which this estimate falls short of the total gross mutation rate is the elimination that must occur at the time of transplanting. Here selection is very rigid against any cytogenetic condition that produces weak seedlings.

Although this figure is admittedly underestimated, the actual total rate probably falls far below the rate of one per cent in Drosophila (TIMOFÉEFF-RESSOVSKY 1937) and of five per cent in Antirrhinum (BAUR 1924). This low mutability is in keeping with the high genetic stability observed by LINDSTROM (1941) in attempts to modify by selection the homozygous diploid tomatoes derived from a haploid. Although low, this mutation rate is sufficient to warrant the search for mutations of a specific kind such as the male-sterile types described above. Mutations occurring with this frequency could inter-

fere with the maintenance of pure lines and should be anticipated in work on the genetics and breeding of tomatoes.

SUMMARY

Gametic sterility is largely responsible for the unfruitfulness of 66 plants discovered in approximately 55,000 field plants of three tomato varieties. Failure of normal gamete formation was resolved to a cytogenetic basis in every case investigated.

Of the 66 unfruitful plants, 45 are triploid, 14 diploid, three tetraploid, two trisomic, and two haploid. Tetraploids are the most fruitful of the heteroploid plants, reflecting greater chromosome balance in gamete formation than in the other types. The majority of heteroploid plants are not found as members of multiple seedlings at germination.

The 14 diploids consist of the following:

1. Three plants aberrant in gross morphology. Sterility of these is probably conditioned either by a pleiotropic effect of the gene determining the deformity or by the deficiency of a chromosomal segment which includes a gene affecting the morphological character and another affecting sterility.

2. Three plants exhibiting male sterility, in each case determined by a single recessive gene.

3. Five plants normal in every respect except for complete pollen and ovule sterility. Failure of viable gamete formation in one plant results from asynaptic meiosis.

4. Three diploids lost during the investigation.

Gene mutation in the broad sense is probably responsible for the sterility of the 11 investigated diploid plants, for reasons discussed in the test. A gametic mutation rate of approximately 0.02 per cent was estimated from these findings.

The three tomato varieties are very similar in the frequency of all unfruitful types and in the proportions of each type. The relative proportions of the discovered types of cytogenetically conditioned sterility in the three varieties investigated here and in other varieties studied to a lesser extent are considered to be representative of most horticultural varieties of the tomato and perhaps of the species as a whole.

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SUGGESTIVE EVIDENCE FOR DUPLICATE GENES IN A SPECIES HYBRID IN DOVES[1]

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FOLLOWING the use of cellular and serum antigens in studying genetic interrelationships of species of pigeons and doves, it has been shown that differences between species in these characters are readily explained according to current genetic theory. Leading references to various reports of this work have been cited in a recent review (IRWIN and CUMLEY 1943). Since antigenic substances are undoubtedly either proteins or more simple structures linked to proteins, the inference seems valid that such studies portray at least a part of the chemical similarities and differences between species, and between individuals within a species.

The antigenic characters of the blood cells have been proposed to be more or less direct products, or at least primary products, of their causative genes (HALDANE 1935; IRWIN and COLE 1936a). However, since the interaction of genes to produce certain antigens in some species hybrids (the so-called hybrid substance) (IRWIN 1932; IRWIN and COLE 1936b; MCGIBBON 1944) and within a species (THOMSEN 1936) is used to explain the experimental results, it may be concluded that more than one step from gene to antigen is sometimes, if not always, involved.

This paper deals with the division of the "hybrid substance," found in cells of the species hybrids between Pearlneck (*Streptopelia chinensis*) and Ring dove (or Ringneck dove, *St. risoria*), into constituent parts in backcross hybrids, and the association of these different parts with antigens peculiar to Pearlneck as compared with Ring dove. There are thus two kinds of antigens under consideration; (a) those which in the various hybrids are presumably produced by the interaction of genes coming from each species, and (b) those which are specific to Pearlneck in comparison with Ring dove. The latter kind are those which are more or less direct products of their respective genes.

MATERIALS AND METHODS

The detection of the hybrid substance is at present possible only following the absorption of antiserums (from rabbits) to the corpuscles of the species hy-

[1] Paper No. 343 from the Department of Genetics, UNIVERSITY OF WISCONSIN. This investigation was supported in part by grants from THE ROCKEFELLER FOUNDATION, and from the WISCONSIN ALUMNI RESEARCH FOUNDATION. An abstract has been published (IRWIN and CUMLEY 1942) of a report presented before the GENETICS SOCIETY, December 1941.

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[3] The species and backcross hybrids whose cells were used in these experiments were produced under the supervision of PROFESSOR L. J. COLE, assisted by DR. N. L. CUTHBERT or DR. RAY D. OWEN. Technical assistance in carrying out the immunological tests was given by MR. WARREN G. BLACK and DR. W. H. MCGIBBON. Grateful acknowledgment is made of the assistance given by each of these.

brid, with the cells of both Pearlnecks and Ring doves. In some tests it was possible to use in absorptions, and in agglutination tests for complete absorption, the cells of either or both parents of certain of the species hybrids, with the same results noted as for tests in which the corpuscles of other individuals within the parental species were used. When all the antibodies reacting with the cells of the parental species are removed from the antiserum, it becomes a "reagent" specific for the hybrid substance. Similarly, the several antigens particular to Pearlneck are detected by the use of Pearlneck antiserum which has been absorbed with Ring dove cells, with further absorptions to determine the identity of birds carrying any one of the different antigens (IRWIN 1939). The details of the more or less standard procedures followed in the production of antiserums and in the absorptions and subsequent agglutinations have been given in previous papers (IRWIN and COLE 1936a; IRWIN, COLE, and GORDON 1936), hence only part of them will be repeated here.

The antiserums were stored at ice box temperature, usually 2° to 5°C, with merthiolate added as a preservative. For the absorptions, the antiserum was diluted 1:30 or 1:60 and mixed in four successive absorptions with a given quantity of cells, depending upon the amount necessary to remove the antibodies for the absorbing cells. For example, from 2.5 to 3 cc of cells from Pearlneck and Ring dove in approximately equal proportions were required to exhaust 1 cc of an anti-hybrid serum (at a dilution of 1:60) of antibodies for the corpuscles of these two species. The agglutinations were done by adding one drop of a 2–2.5 per cent suspension of blood cells to 0.1 cc of the antiserum or reagent in small Pyrex tubes. Readings of the presence or absence of agglutination were made after the tubes had stood at 31°C for two hours, and again after standing overnight in the refrigerator. All negative readings recorded in this paper were checked by microscopic examination. Also, either the third or fourth absorption was allowed to stand overnight in the refrigerator, so that "cold agglutinins" did not complicate these readings.

At the time that the tests reported in this paper were made, it was the ordinary laboratory practice to use the absorbed sera within a week after the absorption was completed. Likewise, reactions with the cells were not considered to be trustworthy unless the suspension of cells was free of lysis. The usual procedure was to make the absorptions of antibodies the first part of the week, and to use the reagents as quickly as possible with cells held in storage no more than a week, generally only two or three days.

Species hybrids have been produced in this laboratory only from matings of Pearlneck males to Ring doves. All the backcross hybrids from matings to Ring doves, in which the cellular antigens particular to Pearlneck have separated and been obtained in unit form (IRWIN 1939), trace back to a single Pearlneck male. However, other matings of Pearlneck males to Ring doves have resulted in species hybrids, of which more than 20 have been tested at various times for the presence of the one or more components of the hybrid substance. Although critical tests of the cells of these hybrids have not been made with each antiserum to determine individual variation of the hybrid substance in the species

hybrids, no differences have been noted among them in antigens peculiar to Pearlneck with which the hybrid substance has been associated. Hence it seems very unlikely that there was any variation among the species hybrids tested, 29 in all, in their content of constituents of the hybrid substance. Repeated tests, over a period of many years, on the cells of several of these species hybrids have given not even a suggestion of a change during that time in reactivity to many kinds of reagents. This statement is based, not upon differences in degree of reactivity from time to time of the cells of an individual, but upon the presence as compared with an absence of reaction of the cells with the same kind of reagent.

EXPERIMENTAL RESULTS AND DISCUSSION

The most reasonable genetic explanation of the production of the hybrid substance complex is that it is effected in the species hybrids by the interaction of genes, each of which in the parental species (Pearlneck and Ring dove) produces only antigens specific to the species. One cannot, of course, ignore the possibility that the genes which interact in the species hybrid to produce the hybrid substance may have no effects except when in combination in the species hybrid—that is, none within the species. However, it has been noted (IRWIN and COLE 1936; and in unpublished material) in certain families (sib-groups) of species hybrids, that none of them contained *all* the antigens of either parental species, although seemingly no differences were observable among the hybrids themselves. Hence a plausible explanation of this peculiar situation would be that certain genes in either species produced species-specific effects, but that these interacted in the species hybrid to give only part or parts of the hybrid substance.

Following the separation of the antigenic complex peculiar to Pearlneck into antigens behaving as hereditary units (IRWIN 1939), it was possible to determine with which units the hybrid substance was associated. There are nine or more antigens which distinguish Pearlneck from Ring dove and which usually behave as genetic entities. These are called d-1, d-2, d-3 . . . d-11 (IRWIN 1939). Of these, d-8 has never been obtained in unit form and, in tests with a limited number of offspring of a bird containing d-4.d-8, was found only in combination with d-4. Also, an immunological distinction between the d-4 and d-9 substances is made only with difficulty, and perhaps these are the same antigen. Possible differences in reactivity of the hybrid substances associated with d-4 and d-9 are presented in this paper and give some evidence of a genetic, if not an antigenic, difference between them.

Two additional antigens specific to Pearlneck are included in this paper—namely, d-10 and d-12. These were both detected as probable units shortly before the time that the tests to be described in this paper were made. The d-10 component appears to be similar to, if not identical with, the d-6 component, but differs in that a part of the hybrid substance has been found associated with it. The d-12 character is immunologically different from any of the others now definitely recognized as units. But whether it is distinct from d-8 is not

known, since the d-8 character was not available for the necessary cross-absorption tests which alone can establish these as separate entities. The backcross progeny of birds carrying either of these two cellular antigens (d-10 and d-12) fulfill the requirements of a genetic unit—that is, half contain the character, and half do not. It was stated in a previous article (IRWIN 1939) that the d-7 antigen was made up of two parts. It now appears that d-3 is probably one of these parts, but to date no offspring have been obtained from backcross hybrids supposedly carrying d-7 alone. However, in this paper d-7 will be dealt with as a distinct substance of Pearlneck, although the fraction of the hybrid substance associated with it may have this relationship by virtue of the d-3 character.

It has been assumed in this work that the genes producing the respective Pearlneck characters are genetically independent. An experimental test of the possibility of linkage between any two or more of them was not possible among the birds of the first backcross generation (IRWIN and COLE 1936a), because most of these were dead when the antigens were recognized in unit form. Occasionally progeny have been obtained from backcross birds carrying two characters specific to Pearlneck, and the segregation in the few offspring obtained from any one mating has been of the four antigenic types anticipated (that is, both characters occurring together, each singly and both absent) in approximately equal numbers. Furthermore, a comparable segregation, also indicating independence of the species-specific characters, has been observed in backcross offspring of birds which had three such cellular antigens. In fact, the segregation noted in all the backcross progenies more closely approaches that expected if random assortment of the antigens prevailed rather than if there were linkage of the causative genes. In an early paper (IRWIN and COLE 1936a), the occurrence of many birds in the first backcross generation which gave serological reactions comparable to those of the species hybrids was interpreted as suggesting a grouping of the causative genes, but later this was shown (IRWIN 1939) to be readily explained as being due to the presence of d-11 in these birds. What evidence is available at present is strongly indicative of these genes for the specific antigens of Pearlneck being genetically independent. However, the possibility of loose linkage between at least certain of them cannot be excluded.

In a previous paper (IRWIN 1939) it was shown that the hybrid substance had been associated with the d-4 and d-11 characters of Pearlneck, and probably with the d-3 antigen. (Antibodies to the d-3 antigen are rarely produced in Pearlneck antiserums, and for years the only reagent for its detection was made from an antiserum to Senegal (*St. senegalensis*). Since absorption of this antiserum by the cells of both Pearlneck and Ring dove did not remove all the antibodies for backcross birds carrying the d-3 substance, this reaction was considered to be evidence of an association of a part of the hybrid substance with the d-3 character. Also, the reagent for the hybrid substance reacts with Senegal cells, and a part of this reaction may be by virtue of the presence in Senegal corpuscles of antigens related to the hybrid substance associated with

the d-3 antigen.) The reagents for the hybrid substance (that is, anti-hybrid serums absorbed by the cells of both Pearlneck and Ring dove), prepared from different antiserums, always reacted with cells possessing the d-11 character and usually, but not always, with those carrying the d-4 component (see second column in table 1). But prior to 1938 no definite reactions of such reagents were noted with cells containing any other antigens of Pearlneck.

TABLE 1

Tests for similarities and differences of the components of the "hybrid substance" associated with various characters specific to Pearlneck.

CELLS	REACTIONS OF THE DIFFERENT CELLS WITH ANTI-F_1 SERUM (NO. 172S1):												
	ABSORBED BY CELLS OF BOTH PEARLNECK AND RING DOVE		ABSORBED BY CELLS OF BOTH PEARLNECK AND RING DOVE IN COMBINATION WITH THOSE OF THE SPECIES HYBRIDS AS LISTED, OR WITH THOSE OF BACKCROSS HYBRIDS WHICH CONTAINED ONE OF THE SEVERAL ANTIGENS SPECIFIC TO PEARLNECK										
	*	172S1	d-1	d-2	d-3	d-4	d-7	d-9	d-10	d-11	d-12	F_1	F_1 Pgn. / R.D.
Pearlneck	o	o	o	o	o	o	o	o	o	o	o	o	o
Ring dove	o	o	o	o	o	o	o	o	o	o	o	o	o
F_1 P.N./R.D.	++	C	++	++	++	++	++	++	++	+	++	o	++
d-1	o	++	o	o	o	±, +	o	o	o	+	o	o	+
d-2	o	++		o	o	+	o			+		o	+
d-3	o, ±	++	o	o	o	o, ±	o	o	o	+	o	o	±
d-4	+	±, +[1]		(o)	(o)	o	(o)			(o)		o	±
d-5	o	o	o	o	o		o		o		o	o	
d-6	o	o											
d-7	o	++, C	o	o	o	+	o	o	o	+	o	o	+
d-9	o	++	o	o	o	o	o	o	o	±	o	o	+
d-10	o	++	o	o	o	+	o	o	o	+	o	o	±
d-11	++	C	++	++	++	++	++	++	++	o	++	o	+
d-12		++	o		o		o	o	o	±, +	o		+
F_1 Pgn./R.D.	+	++	+	++	++	+	+		+	o	+	o	o

Symbols: "C" = complete agglutination; ++ = marked agglutination; + = agglutination; ± = definite but weak agglutination; ± = doubtful reaction; o = no agglutination—all at the first dilution of the serum-cell mixture.

* Other antiserums to species hybrid cells.

[1] The reaction was definite when the antiserum was fresh; after the serum was 15 months old, the reaction was either very weak or entirely absent.

However, in the summer of 1938 an antiserum (#172S1) to the cells of species hybrids was produced which contained very strong antibodies for the hybrid substance. For example, other reagents for this substance ordinarily reacted with hybrid cells at the third dilution—that is, if the first or absorbing dilution was 1:30 or 1:60, the last trace of agglutination usually was noted at a dilution no higher than 1:120 or 1:240. But reagents for the hybrid substance prepared from this antiserum (#172S1) reacted strongly with hybrid cells at a dilution of 1:480, and the last trace of clumping was usually seen at 1:1920 or 1:3840. Some time after this reagent was first used, it was noted that it reacted not only with cells containing either d-4 or d-11, but with the cells of some birds containing other unit substances specific to Pearlneck. The reactions of the

cells carrying any one of these Pearlneck characters with the reagent for the hybrid substance from this antiserum are listed in table 1. For comparison, the results of tests of these cells with reagents prepared from other hybrid antiserums are also included (second column).

The data in the third column of the table show that the reagent for the hybrid character prepared from antiserum 172S1 reacted not only with cells carrying either the d-4 or d-11 substances of Pearlneck but with seven others as well—namely, the d-1, d-2, d-3, d-7, d-9, d-10, and d-12 characters. Not all individuals carrying any one of these different characters have had cells reactive to this reagent, as will be shown later in the tests for genetic segregation. In contrast, not even the slightest trace of reaction has ever been observed in repeatedly testing this reagent with cells containing either the d-5 or d-6 characters. This antiserum to the species hybrids, however, when absorbed only with Ring dove cells, agglutinated the cells with d-5 to a dilution of 1:1440 or beyond, but reacted only slightly if at all with those containing d-6. Although not given in the table, when this antiserum was absorbed only by Ring dove cells, it gave strong reactions with cells carrying all other unit-antigens of Pearlneck.

(It should be emphasized at this point that the reactions listed in the table are due to a part or parts of the hybrid substance *associated* with these unit-characters of Pearlneck, *not* to the unit characters themselves. The absorption of the antiserums to the hybrids by the cells of both Pearlneck and Ring dove removes antibodies peculiar to either species. Those remaining in the immune serum are specific to the antigens comprising the hybrid substances in that they react not at all with the cells of any individuals of either species tested to date, but agglutinate the cells of all species hybrids and those of certain backcross hybrids. The suggestion has been made that the hybrid substance is not qualitatively different from antigens of the parent species, but is rather a higher concentration of antigens normally possessed by one or both parents. In the opinion of at least one of us (M.R.I.) this proposal has no factual supporting evidence, especially since all data from the immunological side, including those of immunochemistry, indicate that in a comparison of two related antigens, the failure of one to absorb an antibody against the other is by virtue of a structural chemical difference in the antigens being compared. McGibbon (1944) has added definite evidence on this point in species hybrids in ducks, in that he was able to produce antibodies against the hybrid substance in the actual parents of the hybrids carrying this new antigen. Since it is believed that individuals are incapable of producing antibodies against constituents of their own cells, the conclusion from his work seems clear that the hybrid substance is a different antigen.)

Clearly, these results suggested that the hybrid substance as such is a complex of components. Accordingly, in order to test this possibility, various absorptions were made of this antiserum (#172S1), using combinations of cells of Pearlneck, Ring dove and those of backcross hybrids carrying any one of the unit-antigens of Pearlneck. The cells of only those backcross hybrids were used

which were also reactive with the reagent for the hybrid substance. Furthermore, since these reagents have nearly always reacted with the cells of hybrids from the matings of pigeon (*Columba livia domestica*) and Ring dove, the cells of this hybrid (F_1—Pgn./R.D.) were included in the tests. These various test fluids may be termed "fractionated reagents" to distinguish them from the "reagent" for the hybrid substance. The results of agglutination tests of these various fractionated reagents towards the various reactive cells are given in table 1.

At the time these absorptions were made, the hybrid antiserum had been kept in storage (2° to 5°C) for 15 months or longer. Unfortunately, the reactions of the reagent with the hybrid character associated with d-4 cells, which were strong when the antiserum was fresh, had become very weak at the time of these tests. Hence, the lack of agglutination of these cells (d-4) with the various fractionated reagents, as given in the table, cannot be interpreted to mean that these same results would have been obtained had these particular antibodies been potent.

The results summarized in the table, in columns 4 to 14 inclusive, are a combination of tests of these fractionated reagents, made primarily at two different times with an interval of several months between the respective absorptions and tests. In the first test, the cells of backcross birds containing the hybrid components associated with the unit antigens d-2, d-3, d-4, d-7, d-11, and of the F_1—Pgn./R.D. were used with those of Ring dove and Pearlneck in respective absorptions. The several test fluids thus produced were then tested with cells carrying the various characters, as given in the table, except those of d-12. In the second test, cells containing substances d-1, d-9, d-11, and d-12 and those of the pigeon-Ring dove hybrid were similarly used in absorptions, and subsequent agglutination tests were made with the various reagents on cells carrying the respective unit characters of Pearlneck, but from different backcross individuals than were used in the previous test. All the cells carrying the unit characters of Pearlneck had previously been found to be reactive with the reagent for the hybrid substance. In the first test the cells of the bird carrying d-9 reacted only weakly with the reagent for the hybrid substance and not at all with the fractionated reagents. (It was later determined, by means of reciprocal absorptions of Pearlneck antiserum, that the cells of this bird possessed only a part of the d-9 character. Quite probably, then, only a part of the usual content of the hybrid substance, associated with the d-9 character of Pearlneck, was present in these cells, and the absence of a reaction with the various test fluids of the table is not necessarily indicative of what should be expected for the full complement of the hybrid antigen associated with this character.) But in the second test, the cells of another backcross bird possessing d-9 reacted strongly with the reagent for the hybrid substance, and in general the reactions of these cells (having d-9) with the other test fluids duplicated those obtained in the first experiment.

It will be seen in table 1 that the cells containing any one of the Pearlneck characters d-1, d-2, d-3, d-7, d-9, d-10, and d-12, when used with cells of Ring

dove and Pearlneck in absorbing anti-hybrid serum, not only removed the antibodies for themselves but for each of the others of this group with which each reagent was tested. In other words, the hybrid substance associated with any one of these seven antigens peculiar to Pearlneck was indistinguishable from, and presumably identical with, the hybrid substance associated with any other of these otherwise distinct antigenic characters of Pearlneck. It should be stated here, however, that reciprocal absorption of an antibody by these different cells may mean only a close chemical similarity of their antigenic characters—in this case a part of the hybrid substance—rather than identity.

However, as shown in table 1, the absorption of this anti-hybrid serum by any one of these seven different kinds of cells in combination with those of Pearlneck and Ring dove did not remove the antibodies for the hybrid substance found in the corpuscles of the species hybrids, nor for that of birds also carrying the d-11 character of Pearlneck, nor for that of the hybrids between pigeon and Ring dove. On the other hand, it may be noted that, after comparable absorptions, the cells of the hybrids between Pearlneck and Ring dove removed all the antibodies from the hybrid antiserum, as would be expected; those containing only the d-11 character of Pearlneck removed antibodies only for such cells and for those of the hybrid of the pigeon-Ring dove cross; and those of the F_1 between pigeon and Ring dove removed the antibodies only for themselves. Further, absorption by cells containing the d-4 component did not remove the antibodies for any other fraction of the hybrid substance, except possibly for that part associated with the d-9 character.

As stated earlier, the reactions of the cells representing the d-9 Pearlneck character with the reagent for the hybrid substance were very weak in the first test of the analysis of the antigenic complex, therefore allowing no definite conclusions from reactions with fractionated reagents as to differences or similarities of the part of the hybrid substance associated with the d-9 character. The hybrid antigen associated with the d-9 component in absorption removed antibodies for that fraction associated with the d-1, d-3, d-7, d-10 and d-12 characters, but the part combined with the d-4 character did not. Unfortunately for complete accuracy, the agglutinative and absorbing capacities of the part or parts of the hybrid substance associated with these two Pearlneck antigens (d-4 and d-9) are not in entire agreement. That is, the reagent for the hybrid substance, absorbed further by the cells containing the fraction associated with d-4, may or may not contain antibodies for the hybrid antigen associated with the d-9 character. But irrespective of what the relationships between these two antigens and the associated hybrid components may be, they do not alter the main conclusions to be drawn from these data.

These findings indicate the presence definitely of two and probably of three fractions of the hybrid substance in the species hybrids between Pearlneck and Ring dove. What appears to be the major fraction—so-called because antibodies are more readily engendered against it than against the other fraction or fractions, and also because cells containing it have reacted more strongly and at higher dilutions than has obtained for the other part or parts—is

linked with the d-11 antigen of Pearlneck. This part is temporarily called "dx-A." Another fraction (called dx-B) has been found to be associated with the Pearlneck antigens d-1, d-2, d-3, d-7, d-9, d-10, and d-12. And if the differential reactivity of this reagent, and of others prepared from other hybrid antiserums than 172S1, with cells also containing the d-4 antigen is accepted as critical evidence, there is a third hybrid component (dx-C) which is combined with the d-4 antigen of Pearlneck. Indeed, the existence of this third component as a separate entity from the second may practically be conceded, since reagents from other hybrid antiserums which agglutinated the cells also containing the d-4 antigen reacted with none of the others (as d-1, d-2, etc.) with which the second component (dx-B) is associated. Further, the distribution of reacting cells in the first backcross generation—24 reacting to eight not reacting with the reagent for the hybrid substance (see table 3, IRWIN and COLE 1936a)—is evidence of two independent constituents (probably those called dx-A and dx-C) of the hybrid substance. It does not distinguish qualitatively between the two parts, however, except by inference.

It is interesting to note that the strong reaction of the reagent for the hybrid substance with the cells of the species hybrid between pigeon and Ring dove is undoubtedly related, primarily if not entirely, to that part of the complex (dx-A) which is found with the d-11 character. That is, the cells of this other species hybrid are not agglutinated by the antibodies remaining in the fractionated reagent after absorption by the part of the hybrid substance associated with the d-11 character (see column 11 in table 1), but react with all the others. Furthermore, when the cells of the pigeon-Ring dove hybrid are used in absorption of this hybrid reagent, antibodies for the different parts of the complex were not completely removed, although the reaction with the dx-A fraction, in cells also carrying d-11, was much reduced in degree. These results indicate that not all of any one of the parts of the hybrid substance complex (of the F_1 from Pearlneck×Ring dove) is present in the cells of the other species hybrid.

Parallel specificities of the hybrid substance of the hybrids from the pigeon-Ring dove cross have been noted. The reagent for this hybrid substance reacts much more weakly, if at all, with the cells of the F_1-P.N./R.D. than with the homologous cells. Definite reactions have been observed of this reagent with d-11 cells, and possibly with others, thereby corroborating the evidence presented above of the relationships existing between the hybrid substance of the d-11 Pearlneck character and that of the F_1-Pgn./R.D. These will be given in detail elsewhere.

No comparable data are available concerning the number and association of the gene or genes of Ring dove which interact to produce the respective parts of the hybrid substance. Offspring from backcrosses of the species hybrid to Pearlnecks are rarely obtained under our laboratory conditions, so that there is little hope of further information on this point. Among the few progeny produced from such matings, however, there was segregation of the parts of the hybrid substance (presumably dx-A and dx-C) reactive with the reagents

available at that time (IRWIN 1939). It would be expected that a particular gene or genes from Ring dove would interact in the species hybrids with one or more from Pearlneck to produce a definite fraction of the antigen, as dx-A, and that interaction between others would produce a second fraction, as dx-C. Given an environment in which offspring from Pearlneck could be obtained, an answer to this phase of the problem would be anticipated.

Segregation of specific Pearlneck antigens and parts of the hybrid substance

Each of the different parts of the antigenic complex of the hybrid substance was first observed when in combination with certain unit-antigens of Pearl-

TABLE 2

Tests for linkage between cellular antigens specific to Pearlneck and components of the "hybrid substance," following matings to Ring doves of backcross hybrids possessing both specific Pearlneck characters and a part of the "hybrid substance."

SPECIFIC PEARLNECK ANTIGEN IN BACKCROSS PARENT	NUMBER OF FAMILIES	NUMBER OF BACKCROSS OFFSPRING POSSESSING				χ^2	P
		BOTH SPECIFIC PEARLNECK CHARACTER AND HYBRID SUBSTANCE	SPECIFIC PEARLNECK CHARACTER ONLY	HYBRID SUBSTANCE ONLY	NEITHER		
d-1	4	6	5	4	15	10	0.011
d-3	2	5	1	1	1	7.5	0.06
d-4	4	14	0	0	24		
d-7	5	15	9	3	7	8.86	0.034
d-9	3	9	5	1(?)	7	8.73	0.036
d-10	3	4	5	3	4	0.5	0.92
d-11	6	11	0	0	10		
d-12	2	4	0	1	3	5.0	0.177

neck. The question naturally arose as to whether all backcross hybrids possessing the Pearlneck characters would also possess the corresponding fraction of the hybrid substance. A summary of the data bearing on this question is given in table 2.

It will be seen in table 2 that there were but two classes of offspring in the backcross matings of birds carrying either d-4 or d-11—namely, those carrying both the Pearlneck character and the hybrid substance, or lacking both. However, backcross birds carrying d-1, d-3, d-7, d-9, d-10, or d-12 produced offspring of either three or four of the four possible antigenic types—namely, (a) those with both the specific Pearlneck character of the parent and the hybrid substance, (b) those with only the Pearlneck character, (c) those with the hybrid substance only, or (d) those with neither of these two kinds of antigens. (Although a part of the hybrid substance had been found to be sometimes associated with the d-2 antigen of Pearlneck, no offspring have been obtained from backcross matings of such birds.)

Thus it appears that, for the limited number of backcross offspring tested from matings in which both kinds of antigens were involved, there was no separation of the two kinds of cellular characters in matings of birds having either the d-4 or the d-11 antigen. However, there was a definite separation, in matings involving each of the other Pearlneck characters (d-1, d-3, d-7, d-9, d-10, and d-12) of the antigen specific to Pearlneck and the fraction of the hybrid substance (dx-B) associated with it. Furthermore, the association of a fraction of the hybrid substance with both d-4 and d-11 antigens in these birds has been checked, for several of the individuals concerned, by reagents prepared from other antiserums than 172S1, and with complete agreement of the association.

These results lead to the conclusion that there is close if not complete linkage between the genes producing, respectively, either the d-4 or the d-11 characters and the component of the hybrid substance accompanying either of these Pearlneck antigens. There is, however, the possibility that the one or more genes which within Pearlneck give rise to either the d-4 or d-11 antigen have a dual effect when in the species or backcross hybrids; that is, they interact with a gene or genes from Ring dove to produce a particular part of the hybrid substance as well as the specific character, but the latter is thereby reduced in quantity. This supposition cannot at present be tested expermentally, and, although perhaps less plausible than the assumption of linkage of the causative genes, cannot be entirely ignored.

This alternative proposal appears somewhat less probable, however, in the light of the seemingly complete separation of the other specific Pearlneck antigens (d-1, d-3 . . .) and the fraction of the hybrid substance found with each of them. From the existing evidence it seems reasonably certain that the genes for each kind of antigenic character are specific in action or interaction, and, if there is linkage between these genes, the segregation observed was by virtue of crossovers.

An alternative explanation to linkage of genes with the two kinds of antigenic effects would be that within the Pearlneck species there was only one chromosome pair carrying one or more genes which by interaction in the hybrids has produced the portion of the hybrid substance (dx-B) associated with these several antigens of Pearlneck (d-1 · · ·). One pertinent measurement of this possibility is application of the analysis for goodness of fit within the progenies to the equality expected in the four kinds of offspring if independent segregation obtained. The values of χ^2 and the probability that the progeny of each kind of antigen deviated from equality in the four classes are given in table 2. Unfortunately, the small numbers in each class do not allow a definite statement to be made as to whether independence or linkage can best explain the relationship between the causative genes. However, except for the progeny of individuals bearing the d-10 antigen, and assuming linkage, the sum of the non-crossover classes is uniformly greater than the sum of the crossover offspring. Thus, the sum of the non-crossover to the crossover progeny for matings involving d-1 is 21 to 9; in those involving d-3, 6 to 2; in d-7, 22 to 12; in

d-9, 16 to 5 or 6; in d-10, 8 to 8; and in those involving d-12, 7 to 1. Since a closer approach to equality should obtain unless linkage were involved, it seems reasonable to conclude that linkage of genes with these two kinds of effects is somewhat more probable. As stated above, this explanation may not hold for the progeny of birds having the d-10 antigen.

If there is linkage between the genes which produce, respectively, a part of the hybrid substance and each of several antigens specific to Pearlneck, there is one primary conclusion to be drawn. The immunological evidence shows that the part of the hybrid substance (dx-B) associated, respectively, with any one of the Pearlneck antigens d-1, d-2, d-3, d-7, d-9, d-10, and d-12, appears to be indistinguishable from, and presumably identical with, that appearing with any other in this group. Hence, the genes which interact to produce the hybrid substance, and which appear to be linked with those effecting these different species characters, are probably very similar, if not identical, although presumably located on different chromosomes. The genes concerned here might be called "repeats," but differ from the "repeats" proposed by BRIDGES (1935) in that those in Pearlneck are probably on different chromosomes, whereas those in Drosophila are duplications of small segments of one chromosome.

As stated previously, the d-7 complex may have associated with it the dx-B hybrid component by virtue of the d-3 character being a part of the d-7 substance. Also, the data in table 2 suggest that the separation of progeny of backcross individuals bearing both d-10 and dx-B on their corpuscles may be that of independent events. However, even after eliminating the association of the dx-B fraction of the hybrid substance with d-7 and d-10, there still remains its occurrence with d-1, d-3, d-9, d-12, and probably with d-2—that is, with four or five different antigens of Pearlneck. What sort of mechanism might account for this seeming duplication of very similar if not identical genes on different chromosomes is only a matter of conjecture. Were there only two chromosomes concerned, one would immediately think of duplication of a part or all of a set of chromosomes. Any one of several explanations involving either ploidy or chromosomal aberrations, or both, might be invoked to explain these findings.

If it be granted that the data support the inference that genes with identical effects (in interaction) are located on separate chromosomes, there would arise among others the question of whether similar examples of duplicate genes are known in other material. What appears to the authors as perhaps the closest approach to a comparable situation is that of the many genes in maize which give rise to albino seedlings. Some of these genes are located on different chromosome pairs, some are linked. However, this case may be different, in that it is not believed by perhaps the majority of workers in the field that, although the end result of these various genes is the same (that is, albino seedlings), the different genes effect the same step in the synthesis of chlorophyll. Rather is it believed that each such pair of genes acts at a different point from any other in the chain of processes involved in chlorophyll formation, in much the same manner that different steps in the production of wild-eye

color in Drosophila is effected by different genes (for discussion and citations, see BEADLE and TATUM 1941; EPHRUSSI 1942).

SUMMARY

The "hybrid substance" of the species hybrids between Pearlneck and Ring dove has been divided into three components, by virture of results obtained by agglutinin-absorption with the cells of various backcross hybrids. One of these fractions has been found always associated with the d-4 antigenic character of Pearlneck, another with the d-11. A third fraction, detectable to date only with reagents prepared from a strongly reacting hybrid antiserum, seemed to be linked, but not completely, with each of several antigens of Pearlneck. This fraction appeared to be but one substance, or a group of very closely related substances, irrespective of association with different antigens of Pearlneck, these latter presumably being genetically as well as immunologically independent. These results suggest that genes with duplicate effects in interaction are located on several chromosomes of Pearlneck.

There is still another possible, but perhaps less probable, explanation of the association of the third fraction of the hybrid substance with several of the unit antigens of Pearlneck. In this explanation, it would be assumed that this fraction was produced by a gene or genes on but one chromosome of Pearlneck interacting in the species hybrid with one or more genes from Ring dove, and the seeming association with various antigens specific to Pearlneck was then entirely one of chance.

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SEED COLLAPSE FOLLOWING MATINGS BETWEEN DIPLOID AND TETRAPLOID RACES OF LYCOPERSICON PIMPINELLIFOLIUM[1]*

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INTRODUCTION

THE seeds arising from reciprocal matings between tetraploid *Lycopersicon pimpinellifolium* (2n = 48) and the common diploid type (2n = 24) are abortive and non-functional. This behavior appears to be characteristic of crosses between diploids and their respective autotetraploids. BLAKESLEE, BELLING, and FARNHAM (1923), for example, found that most of the seeds resulting from the 4n × 2n cross in *Datura stramonium* were non-viable, and JORGENSEN (1928) observed the same result in matings between tetraploid and diploid races of *Lycopersicon esculentum*. Following the 2n × 4n cross in *Zea mays*, according to RANDOLPH (1935), about 98 percent of the seeds are abortive; and of those which are relatively well filled, only a few are germinable. The 4n × 2n cross in maize gives an even greater proportion of poorly developed seeds, although the germinating capacity of these seeds was found to be somewhat higher than in the case of the reciprocal.

While seed abortion is not the only manifestation of incompatibility between diploids and their tetraploid derivatives, it is probably the most effective barrier to hybridization between these races. BUCHHOLZ and BLAKESLEE (1929) have shown that abnormal pollen tube development prevents fertilization in 2n × 4n matings of *Datura stramonium*. RANDOLPH (1935) found that pollen tube growth is retarded in the corresponding cross in *Zea mays*. The restriction associated with pollen tube growth, however, does not prevent interbreeding between diploids and their respective autotetraploids because it does not apply in the reciprocal cross, 4n × 2n. On the contrary, RANDOLPH (1935) observed in matings of Zea which permitted recognition of the two expected classes of seeds that pollen from the diploid is much more effective in accomplishing fertilization than that from the tetraploid when mixtures of the two pollens are applied to the silks of tetraploid plants.

The incompatibility between 4n races and their diploid progenitors is particularly interesting in that it may arise wholly as a result of the difference in chromosome number. The usual failure of the seeds to attain a germinable

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condition following both 2n×4n and 4n×2n crosses is the more remarkable in view of the normal capacity for vegetative growth of the autotriploid plant which may occasionally result from these matings. Obviously the failure is not a function of triploidy as such but arises from the conditions surrounding the triploid embryo within the seed. The nature of the block to the continued growth of diploid-tetraploid hybrids which is interposed at the seed stage is the subject of the present investigation.

MATERIALS AND METHODS

The diploid and tetraploid races of *L. pimpinellifolium* used in this study were described by LINDSTROM (1932), who generously provided us with foundation seed. Both stocks originated from an inbred strain of the Red Currant variety and hence may be expected to carry approximately the same genes. We are indebted to DR. B. L. WADE, U. S. VEGETABLE BREEDING LABORATORY, Charleston, S. C., for seed of the strain of *Lycopersicon peruvianum* (2n=24) which was employed.

Reciprocal matings between the 2n and 4n races of *L. pimpinellifolium* were made in the greenhouse, the usual precautions being taken to control pollination. Both these stocks also were crossed with *L. peruvianum*, using the latter as pollen parent. The results reported by LESLEY and LESLEY (1943) and by SMITH (1944) indicate that the production of functional embryos following the mating *L. esculentum*×*L. peruvianum* varies somewhat depending upon the *peruvianum* (and, perhaps, *esculentum*) strain used. The *peruvianum* stock here employed had been found at the Charleston laboratory to give only abortive seed in crosses with the common tomato. Since *L. esculentum* is fertile with *L. pimpinellifolium*, it was expected that the 2n *L. pimpinellifolium*×*L. peruvianum* cross likewise would give non-functional seeds. This proved to be the case. The controls used were 2n×2n and 4n×4n crosses in *L. pimpinellifolium*.

The matings made comprise two series, as given below, within each of which it was desired to make comparisons of seed development. The chromosome numbers in cells of the maternal tissue, endosperm and embryo of the seeds resulting from the six matings are shown.

	CHROMOSOME NUMBERS IN SEED		
	MATERNAL TISSUE	ENDOSPERM	EMBRYO
(a) 2n×2n, *L. pimpinellifolium*	24	36	24
2n×4n, *L. pimpinellifolium*	24	48	36
2n *L. pimpinellifolium*×2n *L. peruvianum*	24	36	24
(b) 4n×4n, *L. pimpinellifolium*	48	72	48
4n×2n, *L. pimpinellifolium*	48	60	36
4n×2n *L. peruvianum*	48	60	36

The inclusion in each series of a mating involving *L. peruvianum* as a pollen parent makes possible a direct comparison of seed failure associated with the

2n−4n chromosome difference with that resulting from outcrossing to a distinct species. It would have been desirable to include in series (b) a mating with tetraploid *L. peruvianum*, as male parent. A 4n stock of *L. peruvianum*, however, was not available.

Fruits arising from the matings listed were collected at the following times after pollination: 24, 48, 96, 144, 192, 288 and 384 hours. The fruits were killed in Karpechenko's solution and partially dehydrated by moving them through a series of alcohols up to 70 percent. The seeds were then dissected out and prepared for histological study using the methods earlier described for similar work with other species (COOPER and BRINK 1940). The fruits from a parallel set of matings were allowed to remain on the plants to maturity, at which time they were taken for observations on the frequency of seed and fruit formation.

TERMINOLOGY

For convenience the various classes of seeds under study are designated in accordance with the type of mating from which they have arisen. Thus "2n×2n seeds" are the product of a cross between two diploid *L. pimpinellifolium* plants. Those resulting from mating tetraploid *L. pimpinellifolium* with diploid *L. pimpinellifolium*, the latter being used as the staminate parent, are referred to as "4n×2n seeds," and so on. The seed arising from outcrossing the tetraploid and diploid strains of *L. pimpinellifolium* to *L. peruvianum* are designated "4n×*L. peruvianum*" and "2n×*L. peruvianum*," respectively. Throughout the paper the stock used as the pistillate parent in a cross is written first.

FRUIT AND SEED FORMATION

The data on fruit development are summarized in table 1. All but four of the 63 flowers pollinated in the 2n×2n matings formed mature fruits. The results of these control crosses show that the conditions under which the plants were being grown were favorable for fruit development and that the amount of injury to the flowers in castration was not large. The frequency of fruit development was 90 percent in the 2n×*L. peruvianum* cross. The 2n×4n matings, on the other hand, were barren, the flowers and young fruits all being shed within eight days after pollination. Only about one-half the pistillate flowers used in the 4n×4n matings were represented by fruits at maturity. The initial set was high in this case, but immature fruits were shed at intervals up to 24 days. About 76 percent of the 4n×2n crosses gave rise to mature fruits. The rate of fruiting was high also in the 4n×*L. peruvianum* mating, although counts were not made.

The data on seed formation following the several types of matings are brought together in table 2. The "shrivelled" class comprises the seed-like bodies within the mature fruit which are obviously non-functional. This class is not entirely definitive because of gradations in size of the seed-like structures, and the extent to which it represents the seeds which begin to grow but

whose development is prematurely arrested can only be surmised. It is very probable, however, that the remnants of seeds collapsing during the first few days after fertilization are not large enough to be included in it.

The 2n×2n matings gave 26.8 plump and less than one shrivelled seed per ovary, on the average. Since the number of ovules per ovary averaged 29.4, based on counts of 33 ovaries, this approximates a full set of seed. The 2n×*L.*

TABLE 1

Mature fruit development in matings involving 2n and 4n L. pimpinellifolium and 2n L. peruvianum.

MATING	NUMBER		PERCENTAGE MATURE FRUIT
	FLOWERS POLLINATED	FRUIT MATURED	
2n×2n	63	59	93.6
2n×*L. peruvianum*	62	57	90.3
4n×4n	18	10	55.6
4n×2n	33	25	75.7

TABLE 2

Seed production after matings involving 2n and 4n L. pimpinellifolium and 2n L. peruvianum.

MATING	NO. OF FRUITS	NO. OF SEEDS		AVERAGE PER FRUIT	
		SHRIVELLED	PLUMP	ALL SEEDS	PLUMP SEEDS
2n×2n	59	35	1583	27.4	26.8
2n×*L. peruvianum*	55	777	2*	14.2	.04
4n×4n	12	50	95	12.1	7.9
4n×2n	9	225	1**	25.1	.1
4n×*L. peruvianum*	9	97	140	26.3	15.6

* Both occurred in one fruit; seed large, probably accidental selfs.
** Small.

peruvianum mating, on the other hand, yielded only two plump seeds in 55 fruits. Because these two seeds were large and occurred in a single fruit, it is likely that they resulted from accidental self-pollination. The remaining seeds from this mating were shrivelled and averaged 14.2 per fruit. All the fruits from the 2n×4n mating fell before maturity, as noted above.

The number of plump seeds per ripe fruit averaged only 7.9 for the 4n×4n mating. There were in addition about four shrivelled seeds per fruit. All seeds averaged 12.1, as against 35.2 ovules, per ovary. All of the 226 seeds but one in the nine fruits resulting from the 4n×2n mating were shrunken. The constitution of the single small but plump seed obtained was not determined. The 4n×*L. peruvianum* mating is the most fertile in series (b). Nearly one-half

the ovules gave rise to well developed seeds. The germinability of these seeds has not been adequately tested. One hybrid 4n×*L. peruvianum* plant which was reared, however, made an exceedingly luxuriant growth.

The differences in seed production following the above matings are due in part to non-fertilization of ovules and in part to the failure of fertile ovules to develop into plump seeds. The data in table 3 provide a basis for estimating the relative importance of these two factors. Numerous observations on sectioned tomato material show that the ovule enlarges but little beyond the size attained at the mature embryo sac stage unless it becomes fertile. Hence a

TABLE 3

Average number of ovules per ovary enlarged at 12 days after pollination and plump seeds at maturity of fruit.

MATING	ENLARGED OVULES AT 12 DAYS	PLUMP SEEDS AT MATURITY
2n×2n	31.2	26.8
2n×*L. peruvianum*	34.0	.04
4n×4n	7.3	7.9
4n×2n	21.6	.1
4n×*L. peruvianum*	26.3	15.6

gross estimate of the frequency of fertilization may be made by determining the proportion of ovules which increase in size significantly after pollination. Table 3 shows these values for fruits taken at 12 days. The data are based on nine fruits each except in the 2n×2n mating in which ten fruits are represented. On this basis it appears that practically all ovules of 2n plants are fertilized following application of pollen from either 2n *L. pimpinellifolium* or *L. peruvianum*. The sterility associated with the latter mating consequently is attributable to collapse of the hybrid seeds during development. Comparable data for the 2n×4n mating are not available because these fruits all dropped before 12 days.

Infrequent fertilization appears to be an important factor in the low fertility of the 4n×4n mating. Only 7.3 ovules per ovary, on the average, were enlarged at 12 days. On the other hand, a total of 20 seeds and seed-like structures per fruit were found at maturity. The discrepancy is probably due mainly to the elimination of a disproportionate number of fruits with low seeds numbers by abscission. About three times as many ovules become fertile in the 4n×2n mating as in the 4n×4n combination. The very low net fertility of the 4n×2n cross is evidently due to the abortion of seeds during development. The mature fruits resulting from this mating, like those in the 2n×*L. peruvianum* cross, contain no well developed seeds. The frequency of fertilization likewise is fairly high in the 4n×*L. peruvianum* cross. Furthermore, more than half the fertile ovules form plump seeds, in contrast with the 2n×*L. peruvianum* mating in which all or nearly all the hybrid seeds abort.

The occurrence of non-functional seed-like structures within the fruits which mature following the 4n×2n mating agrees with LINDSTROM's (1932) observations on this cross. LINDSTROM found also that the 2n×4n cross was barren.

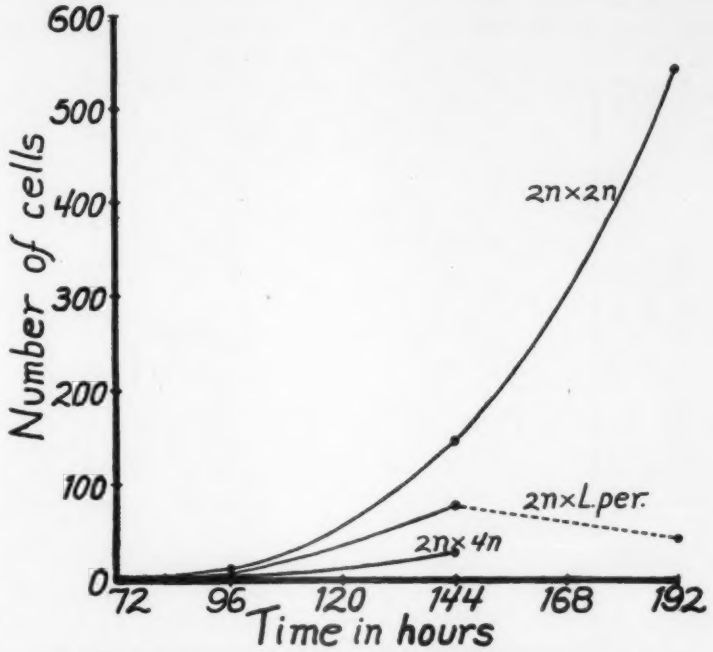


FIGURE 1.—Average number of cells in endosperm following matings involving 2n *L. pimpinellifolium* as the pistillate parent.

RATE OF ENDOSPERM GROWTH

Precocious development of the endosperm is characteristic of the angiosperm seed. *L. pimpinellifolium* conforms to the usual pattern in this respect. Fertilization of the egg and of the central cell are parallel events, but by the time the zygote divides, the endosperm already is a rapidly growing tissue. The ascendancy of the endosperm thus early established is maintained throughout the first stage of seed development. The major phase of embryo growth comes later, although this tissue continues to increase in size steadily, even if slowly, immediately following division of the zygote. Changes affecting the milieu in which the embryo develops occur in the maternal tissues of the seed co-ordinate with, and presumably in response to, the initial activity of the endosperm. It is of primary importance in an analysis of seed collapse, therefore, that early behavior of the endosperm be characterized. Rate of growth of

the tissue at this time is normally high. It appears probable from the results of earlier studies (BRINK and COOPER 1940) and from the tissue relations in *L. pimpinellifolium* to be described below that regular development of other parts of the seed are dependent in a considerable degree upon maintenance of this pace. Accordingly, efforts were made to measure the extent to which rate of endosperm growth varies following the different matings.

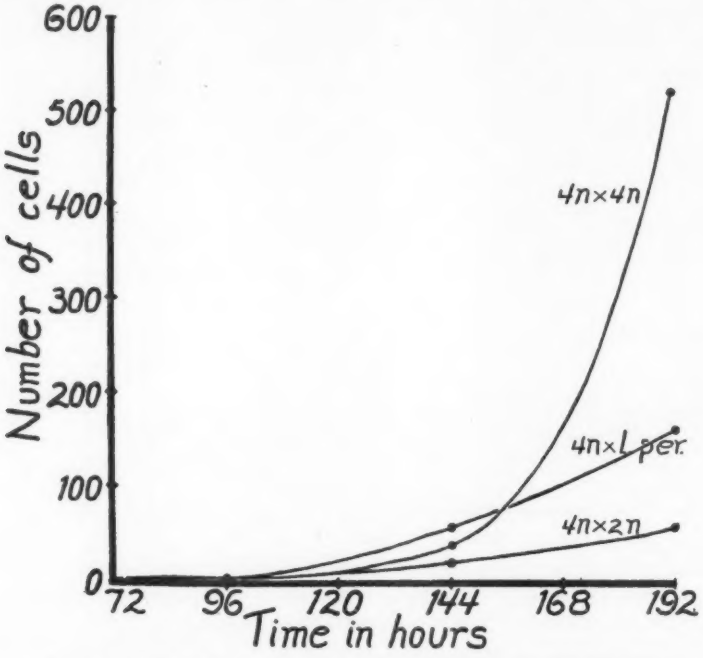


FIGURE 2.—Average number of cells in endosperm following matings involving 4n *L. pimpinellifolium* as the pistillate parent.

Endosperm size, expressed as number of cells, is given in table 4 for the various matings at five periods up to 192 hours after pollination. Cell number was determined from serial sections by counting the nuclei. The endosperm cells are characteristically uninucleate. The 2n×*L. peruvianum* mating at 192 hours, however, is an exception to this rule. Due to a partial breakdown of the tissue, which occurs some time after 144 hours, the parity between nucleus and cell number in the endosperm is destroyed. This unique situation in the 2n×*L. peruvianum* seeds is further described later in the paper.

The data on endosperm size for the matings in which 2n *L. pimpinellifolium* serves as the pistillate parent are plotted in figure 1. It is apparent that the endosperm from the 2n×2n control mating leads the others from the start.

At 144 hours 148 cells are present in the endosperm of the 2n×2n seed, on the average, a value which is twice that for the 2n×*L. peruvianum* endosperm and five times the endosperm size of the 2n×4n seed. The endosperm cell number of the 2n×2n seed increases to an average of 539 at 192 hours. Comparison with the other two classes of seed at this stage cannot be made, because most 2n×4n seeds collapse between 144 and 192 hours, and disintegration of the endosperm begins in the case of the 2n×*L. peruvianum* mating. It is clear from these observations that as compared with normal *L. pimpinellifolium* the

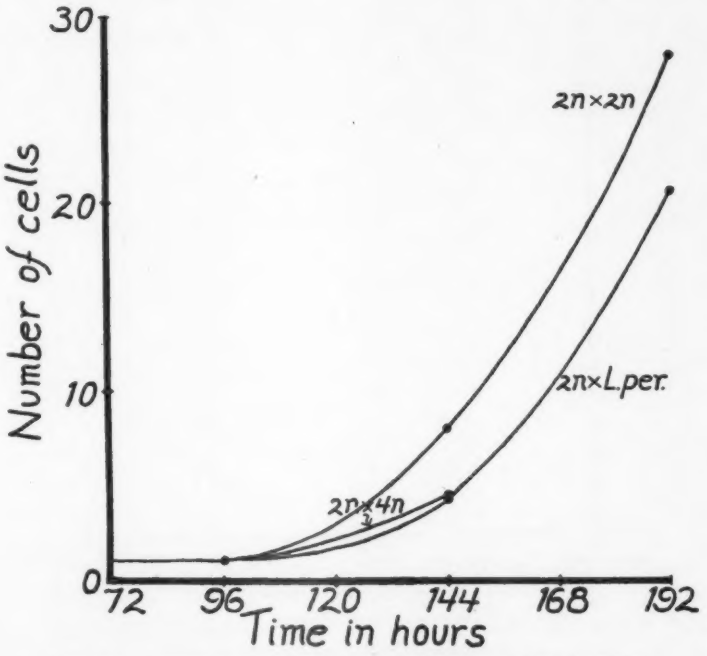


FIGURE 3.—Average number of cells in embryo following matings involving 2n *L. pimpinellifolium* as the pistillate parent.

endosperms formed as a result of the 2n×4n and 2n×*L. peruvianum* matings make a weak growth.

The data from table 4 on endosperm size following the matings in which tetraploid *L. pimpinellifolium* served as the pistillate parent are plotted in figure 2. At 144 hours the endosperm of the 4n×*L. peruvianum* seed is the most advanced averaging 57 cells. The 4n×4n mating is next in order with an average of 36 cells. The endosperms of the 4n×2n seed trail, with a mean of only 20 cells. Endosperm growth in the 4n×4n seeds then accelerates greatly. The tissue was found to have 516 cells at 192 hours, a value which is comparable with that for the 2n×2n seed of the same age. The endosperm of the 4n×*L.*

peruvianum seed continues to grow at an almost steady rate so that at 192 hours the tissue averages 158 cells. It will be recalled from the data presented above that a high proportion of the 4n×4n and 4n×*L. peruvianum* seeds which start to grow are well developed in the mature fruit. The endosperms of the 4n×2n seeds are still very small at 192 hours. Their average size, 55 cells, is about one-tenth the values for the 4n×4n seeds and about one-third that

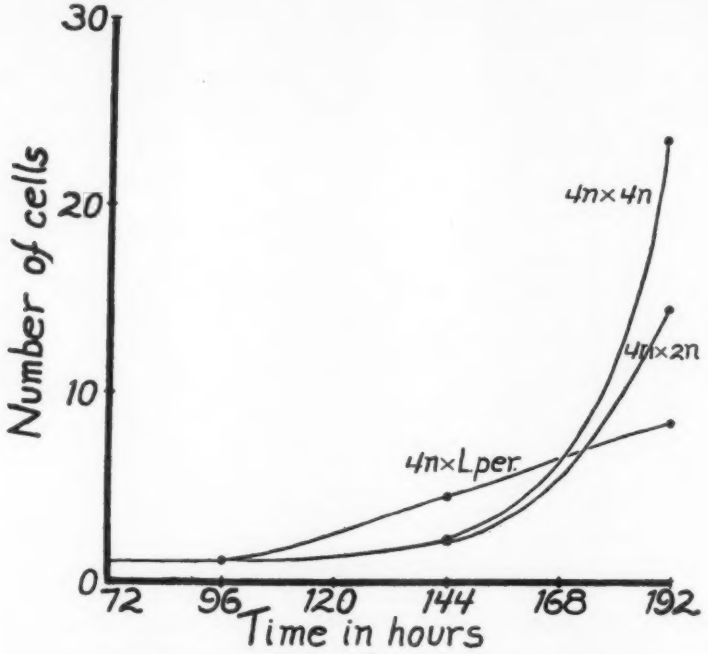


FIGURE 4.—Average number of cells in embryo following matings involving 4n *L. pimpinellifolium* as pistillate parent.

for the 4n×*L. peruvianum* seeds. Unlike these latter two classes, the 4n×2n seeds all collapse before the fruit is ripe.

RATE OF EMBRYO GROWTH

The data presented in table 4 on embryo size for the three matings in which 2n *L. pimpinellifolium* was the pistillate parent are shown graphically in figure 3. It will be observed at once that the numbers of cells in this tissue, particularly at 144 and at 192 hours, are far below those for the corresponding endosperms. Obviously, the growth rate of the embryo at this stage is of a lower order than that of the endosperm.

TABLE 4

Average number of cells in embryo and endosperm at early growth stages after various matings involving 2n and 4n L. pimpinellifolium and 2n L. peruvianum plants.

MATING	NO. OF SEEDS	AVERAGE NO. CELLS	
		EMBRYO	ENDOSPERM
	24 hours		
2n×2n	20	1	1
2n×4n	21	1	1
2n×*L. peruvianum*	14	1	1
4n×4n		not fertilized	
4n×2n		not fertilized	
4n×*L. peruvianum*		not fertilized	
	48 hours		
2n×2n	71	1	1.75
2n×4n	6	1	1
2n×*L. peruvianum*	125	1	1.03
4n×4n	26	1	1.6
4n×2n	138	1	1.2
4n×*L. peruvianum*	50	1	1.06
	96 hours		
2n×2n	200	1	7.8
2n×4n	13	1	4.1
2n×*L. peruvianum*	288	1	3.7
4n×4n	53	1	2.4
4n×2n	306	1	3.5
4n×*L. peruvianum*	191	1	2.3
	144 hours		
2n×2n	25	7.9	148
2n×4n	16	4.4	28
2n×*L. peruvianum*	33	4.2	77
4n×4n	17	2.1	36
4n×2n	56	2.0	20
4n×*L. peruvianum*	30	4.4	57
	192 hours		
2n×2n	4	27.8	539
2n×4n	—	—	—
2n×*L. peruvianum*	21	20.4	(41)*
4n×4n	10	23.4	516
4n×2n	13	14.2	55
4n×*L. peruvianum*	17	8.3	158

* Not comparable; see text.

The most rapidly growing embryos are those in the normal, 2n×2n seeds. These embryos are approximately eight-celled at 144 hours and 28-celled at 192 hours. The embryos of the 2n×*L. peruvianum* and 2n×4n seeds are alike

at 144 hours in being somewhat more than half the size of the controls. The life of the 2n×4n seed is terminated by collapse shortly thereafter. The 2n×*L. peruvianum* seed, however, lives somewhat longer, and the embryo continues to develop, reaching a size at 192 hours about three-quarters of that in the 2n×2n seed.

The data summarized in table 4 on embryo growth in the three classes of seeds resulting from the matings made on tetraploid *L. pimpinellifolium* plants are charted in figure 4. The embryos in the 4n×*L. peruvianum* seeds are the largest at 144 hours, averaging 4.4 cells. The embryos in the 4n×4n and 4n×2n seeds at this time are typically two-celled. This early advantage in embryo size following the outcross to *L. peruvianum*, however, is not maintained. At 192 hours the embryos in the 4n×4n seeds are the largest, approaching in fact the size of those in the normal diploid seeds in the (a) series of matings. The 4n×2n seeds possess more advanced embryos than do the 4n×*L. peruvianum* seeds at 192 hours, the average in the latter case being only 8.3 cells. The non-correspondence between size of young embryo and seed survival in the (b) series of matings is evident. Both the 4n×4n and 4n×*L. peruvianum* seeds in relatively high proportion are capable of developing to maturity, whereas the 4n×2n seeds regularly fail. The latter class of seeds, nevertheless, occupies an intermediate position at 192 hours with reference to embryo size.

ENDOSPERM DEVELOPMENT IN RELATION TO SIZE OF THE ASSOCIATED EMBRYO

Size of endosperm in the different classes of seeds has been considered above in relation to time elapsed after pollination. The data on 144-hour seeds are summarized in table 5 so as to show cell number of the endosperm in relation to size of the associated embryo.

It will be observed from table 5 that, within a given mating, average number of cells in the endosperm increases as the embryo size rises. That is to say, there is a well marked positive correlation in rate of growth of these two tissues in all the classes of seeds.

The effects on endosperm cell number, embryo size remaining constant, of varying the type of staminate parent in matings with diploid and tetraploid *L. pimpinellifolium*, respectively, are of particular interest. Several comparisons are possible in the series of matings in which 2n *L. pimpinellifolium* was used as the pistillate parent. Seeds of this group containing four-celled embryos, for example, average 105 endosperm cells in the 2n×2n mating, 31 in the 2n×4n cross, and 79 in the 2n×*L. peruvianum* mating. The rank is the same for seeds with embryos of the other sizes which are represented in the three different matings. This means that embryos of a given size in the two classes of seeds which collapse during development, 2n×4n and 2n×*L. peruvianum*, are accompanied by smaller endosperms than are embryos in the control 2n×2n seeds which mature normally.

A similar relation is evident in the data relating to the matings in which 4n *L. pimpinellifolium* was the pistillate parent. The seeds from the 4n×2n

cross alone in this series regularly fail, and it is in this mating that the smallest endosperms for a given size of embryo are found.

TABLE 5

Number of endosperm cells associated with embryos of given cell numbers at 144 hours after pollination.

(a) 2n as pistillate parent

NO. CELLS IN EMBRYO	2n×2n		2n×4n		2n×*L. peruvianum*	
	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM
1	—	—	2	15	—	—
2	—	—	3	13	2	45
3	—	—	1	15	3	39
4	1	105	3	31	20	79
5	—	—	2	30	4	91
6	3	140	1	13	3	101
7	7	141	2	41	1	100
8	4	144	1	31	—	—
9	6	162	—	—	—	—
10	4	159	1	80	—	—
Total and average	25	148	16	28	33	77

(b) 4n as pistillate parent

NO. CELLS IN EMBRYO	4n×4n		4n×2n		4n×*L. peruvianum*	
	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM
1	—	—	7	14	—	—
2	16	35	39	21	2	32
3	1	48	10	23	—	—
4	—	—	—	—	13	49
5	—	—	—	—	11	63
6	—	—	—	—	4	77
Total and average	17	36	56	20	30	57

EARLY GROWTH OF THE SEED

The stimulus to growth arising from double fertilization is not restricted to the egg and the central cell within the embryo sac but extends quickly to the contiguous maternal parts of the ovule as well. The following data, based on five seeds in each case, illustrate the large increase in size of ovule which occurs between the mature embryo sac stage and 144 hours after pollination. The values were obtained by measuring the thickness of seeds which had been so

cut on the microtome that the median section passed through both embryo (or egg) and chalazal pocket.

MATING	DIAMETER IN MICRONS	
	MATURE OVULE	144-HOUR SEED
2n×2n	132	271
2n×*L. peruvianum*	132	254
2n×4n	132	211
4n×4n	154	262
4n×*L. peruvianum*	154	272
4n×2n	154	226

The above measurements make it clear that endosperm and embryo do not begin development in a quiescent medium but are required to establish themselves within a relatively large mass of maternal tissue which is simultaneously aroused to rapid expansion.

We have shown in earlier publications how an unbalance of growth between endosperm and integument following enforced self-fertilization in *Medicago sativa* (BRINK and COOPER 1940) and after interspecific hybridization in Nicotiana (COOPER and BRINK 1940) may lead to breakdown of the seed. Since the evidence in these cases suggested that the unbalance might be widely operative in causing seed collapse, close attention was given to the relation between endosperm and maternal tissue in the seeds resulting from the present matings.

TABLE 6

Thickness in microns of the endosperm and endothelium of seeds at 144 and 192 hours.

MATING	THICKNESS IN MICRONS				RATIO ENDOSPERM:ENDOTHELIUM	
	144 HOURS		192 HOURS			
	ENDOSPERM	ENDOTHELIUM	ENDOSPERM	ENDOTHELIUM	144 HRS.	192 HRS.
2n×2n	103	24	156	24	4.3	6.5
2n×*L. peruvianum*	58	41	58	48	1.4	1.2
2n×4n	50	24	—	—	2.1	—
4n×4n	84	24	161	24	3.6	6.7
4n×*L. peruvianum*	86	24	120	24	3.6	5.0
4n×2n	50	34	55	43	1.5	1.3

The data in table 6 show the average thickness of endosperm and endothelium in the six classes of seeds at 144 and 192 hours. Five seeds from each mating in which the median section passed through embryo and chalazal pocket were chosen for measurement. The thickness was computed from the number of 12-micron sections in which the respective tissues were represented in passing across the seed along the plane predetermined as stated. Data on

volume rather than thickness would reveal more accurately the size relations of the two tissues, but the added information thus afforded was not deemed of sufficient importance to justify the greatly increased work involved in determining the third dimension.

Although the data are crude, they clearly reveal a large difference between the viable and inviable classes of seeds at these early stages in relative growth of endosperm and endothelium. This relation is brought out in the last two columns of the table showing the ratio in thickness of the two tissues. The viable classes of seed, 2n×2n, 4n×4n, and 4n×*L. peruvianum*, have large endosperms and uniformly thin endotheliums and, hence, high ratios. The seeds destined, on the other hand, to collapse, 2n×*L. peruvianum*, 2n×4n, and 4n×2n, have smaller endosperms and (with the exception of 2n×4n at 144 hours) thicker endotheliums, with resulting low endosperm-endothelium ratios.

More detailed observations on endosperm and endothelium in the six classes of seeds are presented in the section following.

THE 2n×2n SEED

Fertilization occurs in diploid *L. pimpinellifolium* within 24 hours after pollination. Evidence of the rapid endosperm development following fertilization has been presented in table 4 and figure 1, above. The early changes appearing in the maternal tissues of the seed are illustrated in figure 6, based on 144-hour material. Figure 6 may be compared with figure 5 showing the ovule in median section at the mature embryo sac stage. A large increase in size of the developing endosperm and embryo which arise from the fertile embryo sac and in size of the entire seed occurs during the first six days. Enlargement of the integument results both from cell expansion and increase in cell number by mitotic division. The cells lying between the apex of the single vascular bundle entering the seed and the chalazal pocket become more clearly differentiated into a conducting tissue thus providing a direct nutritive connection between the endosperm and regions outside the seed.

The apical and lateral cells of the nucellus become disorganized and ultimately disintegrate during the course of development of the ovule so that the mature embryo sac is immediately surrounded by the massive integument. Following fertilization the inner epidermis of the integument, the endothelium, becomes differentiated into a well-defined layer of densely-cytoplasmic cells. This endothelium is continuous with the lining of the micropylar canal and surrounds the expanding endosperm except for a small gap at the chalazal end where it assumes a tube-like form leading toward the vascular bundle. It is opposite the gap that the small chalazal pocket in which the conducting tissue terminates is formed before fertilization occurs. The endothelium normally remains one cell in thickness, the cells dividing along the radial axis as the seed grows.

A few to several layers of integumentary cells immediately outside the endothelium lose their cytoplasmic contents during early development of the seed

(fig. 6, 7). The matrix of cell walls persists so that the shape and position of the inner portions of the seed are not altered. The depleted tissue becomes nearly continuous around the endothelium by 192 hours. The distal cells of the conducting strand tend to remain nucleated and retain at least a part of their cytoplasmic contents. The depletion is somewhat less complete, therefore, in the chalazal region than elsewhere.

It is probable that the protoplasts in the depleted part of the living seed are replaced with a liquid which fills the interstices of the matrix. This would mean that the sac-like endothelium enclosing endosperm and embryo is supported in a relatively homogeneous fluid medium, the homogeneity being maintained by the ready diffusion of solutes in the absence of semipermeable membranes.

The integumentary cells which become emptied of their contents serve as an initial source of nutriment for the young endosperm, embryo, and associated endothelium. The main source of food, however, is outside the seed. Access to it is afforded by the vascular bundle. The movement of nutrients into the endosperm appears to take place in part through the chalazal aperture lying opposite the end of the conducting tissue and in part also through the endothelium. It will be seen from figure 6 that the cytoplasm of the endosperm cells opposite the chalazal pocket is relatively dense, a condition suggesting an active rôle in the absorption of nutrients. Frequently the endosperm cells in this region are seen to be elongated in the direction in which nutrients would be expected to move. The peripheral cells of the endosperm, in general, are somewhat more densely cytoplasmic than those of the interior. It is suggested that this condition is associated with the absorption of nutrients from the endothelium which appears to be active in promoting cytolysis in the adjacent integumentary tissue. Food materials entering the seed through the vascular bundle and diffusing into the depleted portion of the integument, likewise, may be absorbed by the endosperm through the endothelium.

THE 2n×L. PERUVIANUM SEED

The frequency of fertilization in the 2n×*L. peruvianum* cross is as high as that in the 2n×2n mating, but all the seeds collapse before the fruit is ripe. The changes leading to failure are of interest in themselves but were studied in the present case principally for the purpose of relating them to the events which terminate in breakdown of the 2n×4n seed.

Growth of the endosperm is retarded compared with that of normal *L. pimpinellifolium*. This fact is evident from the data presented in table 4 and figure 1. Embryo growth in the 2n×*L. peruvianum* seed is slowed down also, but the relative decrease is less than that in the endosperm.

The cells of the endosperm immediately adjacent to the embryo at 144 hours have dense cytoplasm, whereas those at the chalazal end are highly vacuolate and appear starved (fig. 10). As growth continues, the endosperm increases considerably in volume in spite of the fact that there is a decrease in the number of cells. All the cells are large and highly vacuolate at 192 hours (fig. 11,

12). A few of the cells on the dorsal side of the endosperm near the apex of the embryo have become greatly increased in size due to the breakdown of intervening cell walls and the fusion of two or more cells, which accounts for the decrease in cell number. The nuclei of these fusing cells likewise unite so that large nuclei with numerous nucleoli are present. Further enlargement of the cells and continued fusion of those cells in the micropylar region takes place so that by 288 hours the embryo comes to lie in a single enormous cell which occupies the micropylar half of the endosperm, as shown in figure 13. A giant nucleus is present. Continuing dissolution of cell walls and fusion of nuclei is evident at the chalazal end of this cell. This type of breakdown persists until the entire endosperm is comprised of only a very few cells. The endosperm then collapses, and shortly thereafter growth of the embryo and seed ceases.

The effects of outcrossing diploid *L. pimpinellifolium* to *L. peruvianum* on early behavior of the accessory tissues of the seed are illustrated in figures 10 and 11. A conspicuous thickening of the endothelium mainly on the dorsal side of the endosperm near the chalazal pocket is evident at 144 hours (fig. 10). The cells in this region are becoming meristematic. The starved condition of the proximal portion of the endosperm adjacent to the hypertrophied endothelial tissue is significant. Noteworthy also is the accumulation of deeply staining particles in the chalazal pocket. The nature of this material has not been determined. Depletion of the integumentary cells adjacent to the endothelium proceeds at about the same rate as in 2n×2n seeds.

THE 2n×4n SEED

Endosperm development is slow from the start in the short-lived 2n×4n seeds. The data in table 4 show that the tissue is only about one-half the size of the control endosperm at 96 hours and at 144 hours has only 28 cells, on the average, in comparison with 148 cells for the normal endosperm from 2n×2n matings. Growth of the embryo is retarded also.

A marked hypertrophy of the endothelial cells lying along that portion of the dorsal surface of the endosperm nearest the chalaza is clearly in evidence at 144 hours (fig. 16). Depletion of the cells in the inner portion of the integument does not proceed as rapidly as in the 2n×2n seed. As in the 2n×*L. peruvianum* seed, there is a considerable accumulation of stainable material just outside the chalazal aperture.

THE 4n×4n SEED

The developing seed of the tetraploid resembles that of the diploid very closely (fig. 8, 14). Fertilization, which occurs within 24 hours after pollination of the 2n plant, takes place somewhat later in the tetraploid. Growth of the endosperm also starts a little more slowly. Cell number in this tissue, however, is increasing very rapidly at 192 hours, and at this time the size of the tissue is nearly equal to that of the diploid. The embryo of the tetraploid follows a course of growth parallel to that of the endosperm in that it starts slowly but approaches the size of the diploid embryo at 192 hours.

Through differentiation of the intervening integumentary cells the endosperm of the tetraploid early establishes through the chalazal pocket a direct nutritive connection with the vascular bundle of the seed. The extent and rate of the differentiation appears to be slightly greater after the 2n×2n and 4n×4n matings than in the other classes of seeds.

The endosperm cells containing the densest cytoplasm are situated opposite the chalazal aperture and on the surface of the endosperm in contact with the endothelium (fig. 9). The endothelium remains a single layer of cells in thickness as the seed grows; and the cells of the inner portion of the integument gradually lose their contents. The 4n×4n seed is indistinguishable from that of the diploid in these respects.

THE 4n×L. PERUVIANUM SEED

About 60 percent of the ovules which become fertile in the 4n×*L. peruvianum* mating develop into plump seeds. This relatively high net fertility stands in sharp contrast with the early and complete abortion of seeds following the 2n×*L. peruvianum* cross.

The endosperm of the 4n×*L. peruvianum* seed grows more slowly than that of the 4n×4n seed so that at 192 hours it is only about one-third as large (table 4). The regions of densest cytoplasm in the endosperm are opposite the chalazal pocket and adjacent to the endothelium (fig. 15). No evidence of nuclear fusion, such as occurs in the endosperm of the 2n×*L. peruvianum* seed, has been observed in this tissue. The embryo of the 4n×*L. peruvianum* seed is about one-half the size of that in the 4n×4n seed at 192 hours.

The condition of the accessory tissues of the 4n×*L. peruvianum* seed at 144 hours is illustrated in figure 15. The endothelium consists of a single cell layer, although there is a tendency in some seeds for the cells to thicken in the dorsal region near the chalazal pocket. Rarely, however, do these cells divide in the plane normal to the endothelium. The depleted area of the integument is similar in extent to that in the 4n×4n seed.

THE 4n×2n SEED

The tetraploid used as the pistillate parent in matings with the diploid gives a high proportion of fertilized ovules, but the seeds are incapable of developing to a germinable condition.

Growth of the endosperm in the 4n×2n seed is conspicuously weak. The tissue possesses only 55 cells, on the average, at 192 hours (table 4). This is about one-third the number in the 4n×*L. peruvianum* seed and approximately one-ninth as many as occur in the 4n×4n seed at this time. The embryo resulting from the 4n×2n mating is comparable to that of the 4n×4n seed up to 144 hours, but it falls sharply behind at 192 hours. The embryo of the 4n×2n seed at 192 hours, however, is larger than that of the 4n×*L. peruvianum* seed.

Differentiation of the tissue between the apex of the vascular bundle and the chalazal pocket is less complete at 144 hours than in the 4n×4n seed.

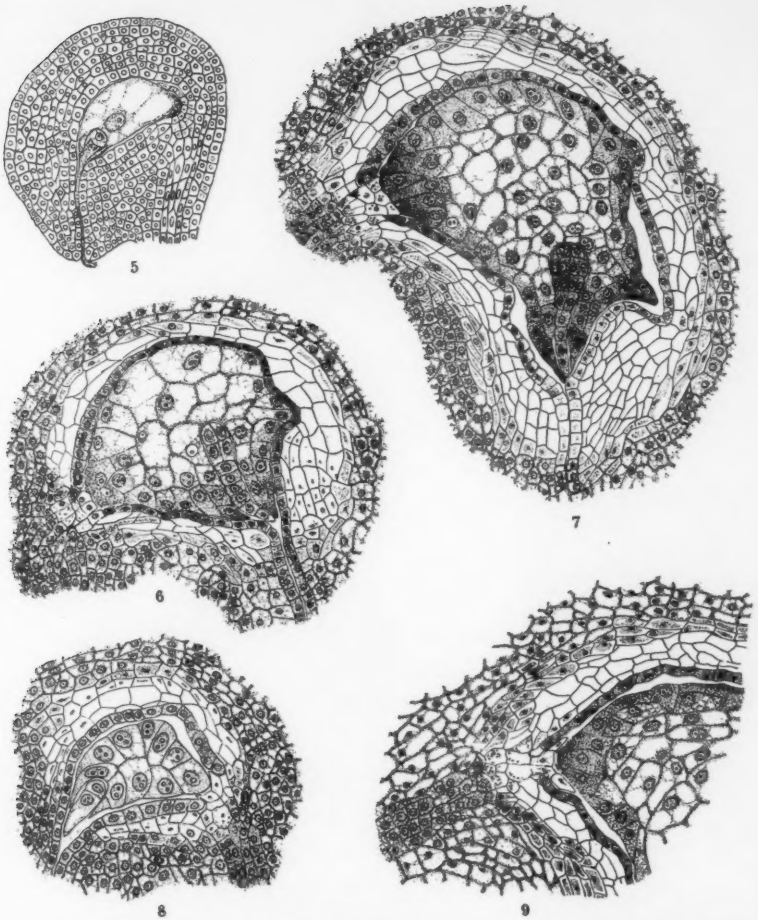


FIGURE 5 TO 7.—2n×2n.—Fig. 5. Ovule at time of fertilization.—Fig. 6 and 7. Endosperm and adjacent tissue at 144 hours and 192 hours, respectively, following pollination.

FIGURE 8, AND 9.—4n×4n.—Fig. 8. Endosperm and adjacent tissue at 144 hrs.—Fig. 9. Chalazal pocket region at 192 hours. ×132. Note the single layer of densely cytoplasmic endothelial cells. Several layers of integumentary cells immediately adjacent thereto lose their contents in the course of development. Compare fig. 6 and 7.

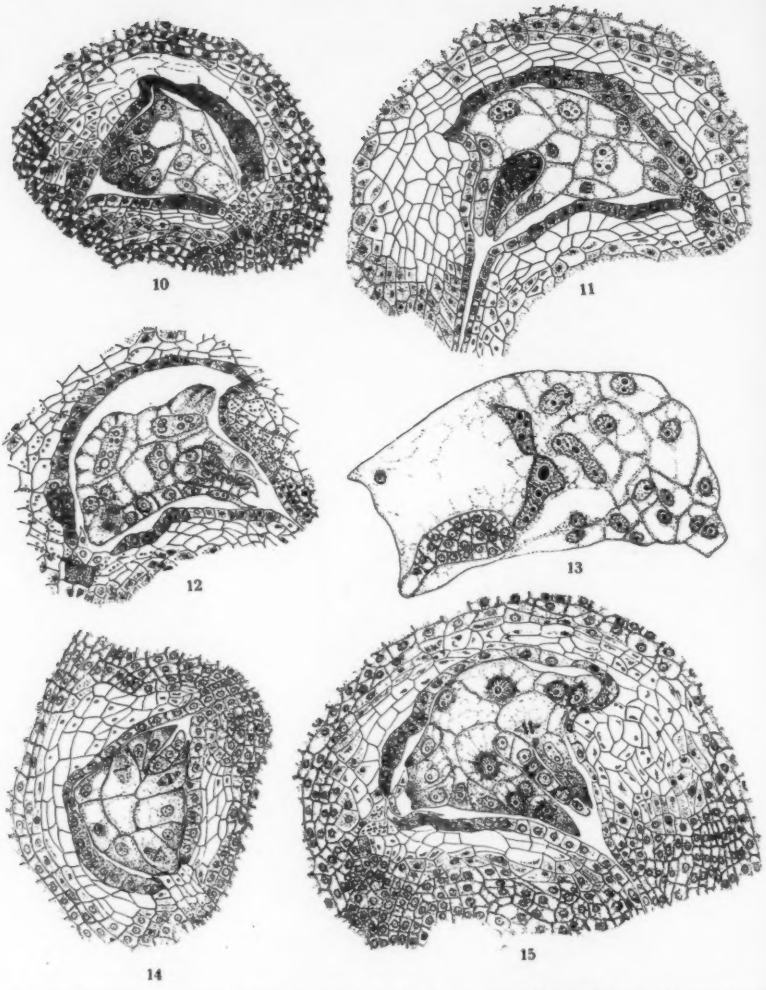
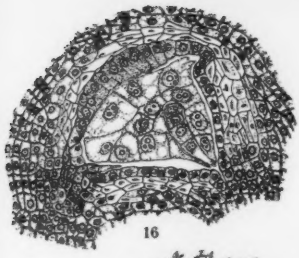


FIGURE 10 TO 15.—2n×*L. peruvianum*.—Fig. 10. Endosperm and immediately adjacent tissue at 144 hours showing starved condition of chalazal cells of endosperm. The endothelial cells on the dorsal surface of the endosperm are greatly increased in size.—Fig. 11 and 12. Same at 192 hours. Some cells of the endosperm contain giant nuclei. The cells of the chalazal pocket are filled with a densely staining material.—Fig. 13. Endosperm and embryo at 288 hours showing the breakdown of those endosperm cells in the region of the embryo and the fusing nuclei. ×132.—FIG. 14 AND 15.—Endosperm and adjacent portion of integument of 144 hours in 4n×4n and 4n×*L. peruvianum*, respectively. ×132.



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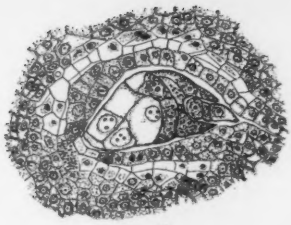

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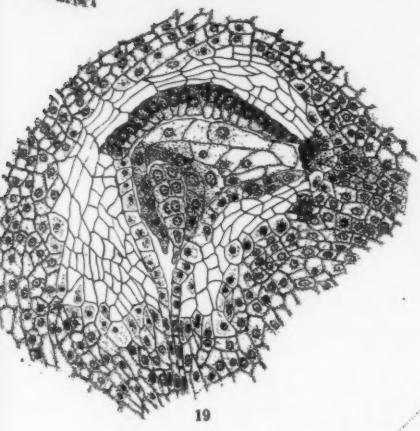

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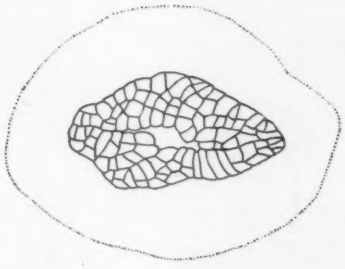

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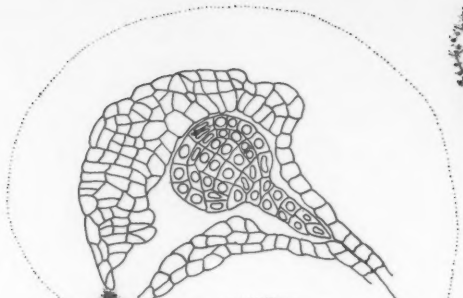
FIGURE 16 TO 19. Endosperm, endothelium, and immediately adjacent cells of integument. ×132.—Fig. 16. 2n×4n, at 144 hours. Note highly vacuolate cells of endosperm and enlarged endothelial cells.—Fig. 17 and 18. 4n×2n at 144 hours showing lag in development of the endosperm and the overgrowth of the cells of the endothelium.—Fig. 19. Same at 192 hours showing meristematic condition of the cells of the endothelium. The cells of the chalazal pocket are filled with a granular material.

FIGURE 20.—4n×2n. Polar view of mitotic figure in the endosperm showing 60 chromosomes. ×1320.

FIGURE 21.—4n×2n. Transverse section through overgrown endothelium at 288 hours. Note small endosperm cavity. The dotted line shows the limits of the cells of the integument which are empty. ×132.



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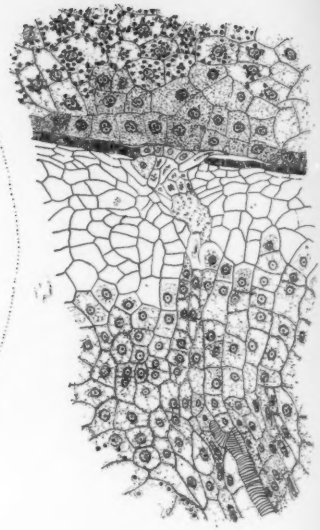

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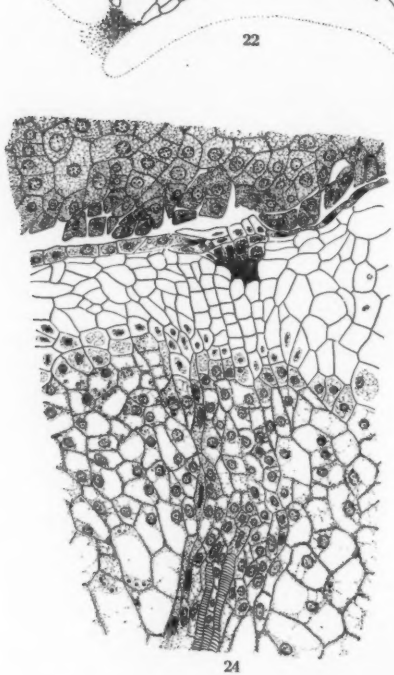

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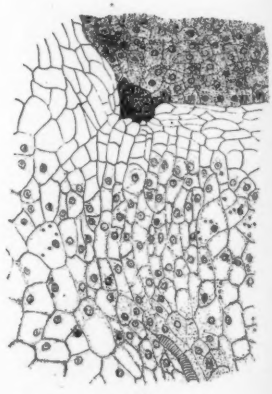

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FIGURE 22.—4n×2n. Embryo and overgrown endothelium at 288 hours. Note the rounded embryo, empty endosperm cavity and densely staining material in chalazal region opposite endosperm cavity. ×132.

FIGURE 23 TO 25.—Longitudinal sections of chalazal portions of seeds at 384 hours showing relationships between endosperm, endothelium, and vascular tissue.—Fig. 23. 4n×4n showing densely cytoplasmic absorbing cells of the endosperm immediately opposite chalazal pocket. Starch is being stored in the highly vacuolate central cells of the endosperm.—Fig. 24. 4n×*L. peruvianum*. The epidermal cells of the endosperm are breaking apart, and no starch is being stored in the central densely cytoplasmic cells. The endothelium persists as a single layer of cells.—Fig. 25. 4n×2n showing extensive hyperplasia of the endothelium. Note that in figures 24 and 25 the cells of the chalazal pocket are packed with a granular material. ×132.

Deeply staining granules accumulate in the chalazal pocket of the 4n×2n seed and are frequently seen also within the endosperm at 144 hours (fig. 17, 18). If these particles are reserve food of some sort, they nevertheless do not insure against starvation of the endosperm. The chalazal portion of the endosperm may become depleted nearly to the point of collapse in spite of an abundance of the granules in nearby cells (fig. 17). Evidently some substances other than those in the deeply staining granules are in short supply in the starving tissue.

Hyperplasia of the endothelium in the 4n×2n seed is apparent at 144 hours (fig. 17, 18). Proliferation of the cells begins near the chalaza in the dorsal region of the endothelium. Figure 19, based on a 192-hour seed, shows the hyperplastic portion of the endothelium extending dorsally from the chalaza to a point opposite the anterior end of the embryo. The cells of the endosperm adjacent to the overgrown portion of the endothelium are very poor in cytoplasm. The endosperm has broken down entirely by 288 hours, and the embryo is surrounded by a mass of overgrown endothelial tissue, as illustrated in figures 21 an 22.

THE SEVERAL CLASSES OF SEEDS AT 16 DAYS

The 2n×2n mating and two of the matings on tetraploid plants, 4n×4n and 4n×*L. peruvianum*, yield a high proportion of viable seeds. A few seeds from the 2n×*L. peruvianum* and 4n×2n crosses persist for 16 days, although this is about the limit of their life span. It is of interest to examine the histological condition of these seeds at the time of their ultimate separation into two classes on the basis of viability.

The relation of the tissues in the chalazal region of the 4n×4n seed is depicted in figure 23. Considerable storage of granular food reserves in the endosperm has occurred. The outer layer of endosperm cells, and the cells in the region opposite the chalazal aperture, however, are densely cytoplasmic and free of visible storage materials. Apparently the main function of these cells is absorption of nutrients from the solution with which the cell wall matrix surrounding the endosperm presumably is filled. It will be noted from figure 23 that the conducting tissue distal to the vascular bundle is well organized in the form of rather regular columns of cells leading toward the chalazal pocket region.

The most conspicuous differences in the 4n×*L. peruvianum* seed at this time, as illustrated in figure 24, appear in the endosperm. In the first place no granular reserves are found in this tissue, which is rather densely cytoplasmic in the entire outer portion. Secondly, the endosperm cells adjacent to the endothelium are separating from each other. This partial disorganization may result from growth of the endothelium at the expense of the endosperm protoplasts in the absence of food reserves. There is a considerable accumulation of deeply staining material in the chalazal pocket. The conducting tissue at the apex of the vascular bundle is somewhat less clearly differentiated from the surrounding cells than is the case in the 4n×4n seed.

The endosperm of the 4n×2n seed has disappeared entirely by 16 days, and the space which it normally occupies has become nearly filled with a densely staining mass of overgrown endothelial tissue (fig. 25). The material of unidentified character which begins to accumulate early in the chalazal pocket in seeds of this class is present in large amounts at this stage. The conducting tissue is similar in appearance to that of the 4n×*L. peruvianum* seed.

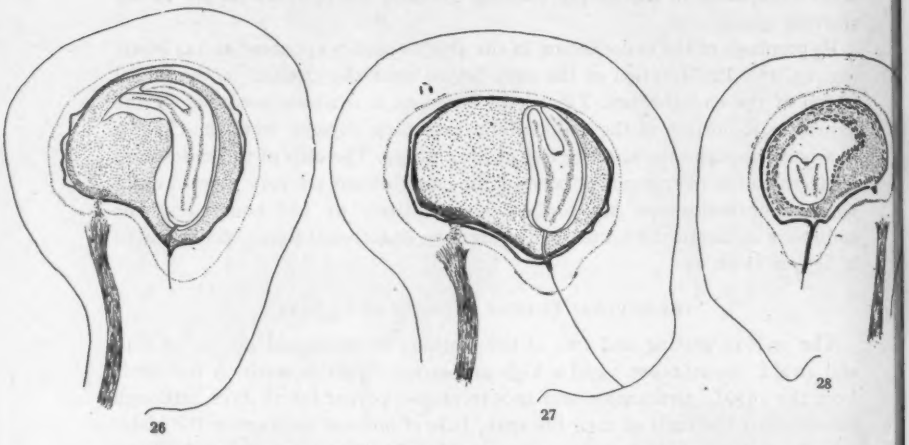

FIGURE 26 TO 28.—Median longitudinal sections through developing seeds at 384 hours showing the relationship existing between embryo, endosperm, integument, and vascular tissue. ×22. The dotted line outside the endothelium shows the limits of the empty cells of the integument (semi-diagrammatic).—Fig. 26. 2n×2n. The embryo with well developed cotyledons grows at the expense of the endosperm. The principal absorbing region of the endosperm is immediately opposite the chalazal pocket.—Fig. 27. 4n×4n. The development is similar to that found in the 2n×2n seed.—Fig. 28. 4n×*L. peruvianum*. Development is much slower than in the 4n×4n seed. The small embryo is differentiating in a normal manner. The peripheral layer of endosperm cells and those adjacent to the embryo are breaking down.

An outline drawing of a median section of the 2n×2n seed at 16 days is shown in figure 26. The embryo, imbedded in the endosperm, shows prominent cotyledons and a well defined vascular tissue. It is apparent from figure 27 that the 4n×4n seed of the same age is closely comparable. Gross seed size is not greatly different, the extent of depleted tissue, the boundary of which is indicated by a dotted line, is about the same, and in both seeds the endothelium is a thin and relatively inconspicuous tissue. The embryo of the 4n×4n seed is slightly smaller than that of the diploid at this stage.

The 4n×*L. peruvianum* seed at this time is definitely smaller than its 4n×4n counterpart (fig. 28). As shown in detail in figure 24, the outer cells of the endosperm are becoming separated from each other, and this tissue is being digested in the boundary zone of the relatively large cavity in which the embryo lies. The embryo is much less advanced than that in the 4n×4n

seed, although normal in form. The data in table 2 show that about one-half the fertile ovules from the 4n×*L. peruvianum* mating develop into plump seeds. Figure 28 is illustrative of the best developed seeds of this class at 16 days.

DISCUSSION

The results, in terms of seed production, of the four *L. pimpinellifolium* matings reported upon here agree with the findings of other investigators in showing that autotetraploids are fertile *inter se*, like diploids, but that reciprocal crosses between tetraploids and diploids are sterile, or nearly so. The tetraploid tomato produces considerably fewer seeds than the diploid, but this is due mainly to failure of many ovules to become fertile because of restricted pollen tube growth rather than to breakdown of seeds during development.

The course of seed development following the 4n×4n mating agrees in detail with that of diploid *L. pimpinellifolium*. The endosperm of the tetraploid starts growth more slowly, but by eight days it is on a par with that of the diploid. A similar initial lag characterizes the tetraploid embryo also. The endothelium, a single layer of specialized cells investing the endosperm, behaves regularly in the 4n×4n seeds as does the adjacent integumentary tissue, which becomes depleted of its protoplasmic contents. The relationship established between the vascular bundle entering the seed and the chalazal pocket, which lies opposite a small aperture in the endothelium, by differentiation of the intervening cells into a conducting tissue, is essentially the same in the tetraploid as in the diploid seed. In kind and sequence alike the histological changes involved in transformation of the fertile ovule of the tetraploid into a normally developed seed are indistinguishable from those in the diploid.

When, however, the diploid is substituted for the tetraploid as staminate parent in the mating with the tetraploid or, *vice versa*, the tetraploid is used as staminate parent in crosses with the diploid, the behavior of the resulting fertile ovules is quite different. Both the 4n×2n and 2n×4n seeds fail to develop to a germinable condition. Significant also is the fact that the course of events leading to collapse, so far as it can be discerned histologically, is the same in the two kinds of seeds. The first detectable departure from normal behavior, as exemplified by the respective 4n and 2n controls, is a decreased rate of endosperm growth. Table 4 shows that at the three periods beyond fertilization at which observations were made—namely, 48, 96, and 144 hours—the endosperm of the 2n×4n seed contains fewer cells than the endosperm of the 2n×2n seed. The difference in endosperm size increases with age of the seed, becoming five-fold at 144 hours. Although the 96-hour seeds do not conform, the same general relation holds in comparing 4n×2n and 4n×4n seeds. The endosperm of the latter is nearly twice the size of that of the former at 144 hours and over four times the size at 192 hours. It is evident from table 5 also that for a given size of embryo the number of endosperm cells is regularly greater for 2n×2n than for 2n×4n seeds and for 4n×4n than for 4n×2n seeds.

The endothelium in 2n×4n and 4n×2n seeds departs at an early stage from the course of development followed by the diploid and the tetraploid. The difference is discernible in some seeds at 96 hours and becomes pronounced by 144 hours. The endothelium in the control seeds comprises a single layer of cells flattened against the endosperm which it surrounds. Growth of the endothelium by cell division is coordinate with that of the enlarging endosperm, the latter being the more aggressive tissue. It would appear that in the normal seed the size of the endothelium is merely accommodated to that of the rapidly expanding endosperm, to which it is subordinate. This nicely adjusted balance is upset very early in 2n×4n and 4n×2n seeds. The sequence of abnormal developmental changes which then follows leads directly, even if not always quickly, to collapse of the entire seed.

The first visible evidence of atypical growth of the endothelium is a thickening of the cells in the dorsal region near, but not immediately adjacent to, the chalazal pocket. As these cells become increasingly meristematic in appearance they orientate themselves with their long axes vertical to the endosperm surface. The long axis thus comes to lie at right angles to the normal position. Clear evidence of polarization of the cells in this direction is sometimes seen (fig. 19). The endosperm cells just beneath the over-active part of the endothelium become thinly cytoplasmic and starved in appearance. The evidence afforded by these tomato seeds leaves no room for doubt that weak growth of the endosperm and hyperplasia of the endothelium are intimately related phenomena.

Overgrowth of the endothelium continues and is accompanied by further impairment of the endosperm. Eventually the endosperm is entirely destroyed. The embryo, already much retarded in growth and obviously undernourished, although not yet beginning to disintegrate, now lies in a small cavity surrounded by a large mass of activity proliferating endothelial tissue. The embryo may continue to live for several days under these conditions. Growth soon ceases, however, and the entire seed collapses.

The course of developmental events leading to breakdown of the 2n×4n and 4n×2n *L. pimpinellifolium* seeds parallels in all essential features that which the writers have described for certain interspecific hybrid seeds in Nicotiana (Cooper and Brink 1940). Weak growth of the endosperm and overgrowth of the adjacent maternal tissue characterize the seeds which fail in both these cases. The embryo dies not because it is inherently defective, but as a result of disturbances in the accessory tissues of the seed which render the latter an unfavorable medium for continued growth of the embryo.

The close correspondence between seed failure associated with species hybridization and with crosses between diploids and tetraploids of the same species is further attested by the behavior of the 2n *L. pimpinellifolium*×*L. peruvianum* mating, as described above. The endosperm of the young seeds from the tomato species cross grows slowly like that resulting from the 2n×4n mating, and the endothelium becomes hyperplastic in the same way and at the same time. Sansome, Satina, and Blakeslee (1942) have observed that the

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inviable seeds of *Datura stramonium*×*D. metel* and *D. stramonium*×*D. ceratocaula* likewise behave much as those from the 4n×2n mating in *D. stramonium*.

The breakdown of endosperm cell walls accompanied by the formation of giant nuclei in the vicinity of the embryo observed at 192 hours after the 2n×*L. peruvianum* mating, however, does not have a counterpart in the 2n×4n seeds. Disintegration of the endosperm in the vicinity of the embryo appears to be a premature manifestation of the parasitism which is normal to the relationship between these tissues. The early onset of cellular dissolution in this region indicates an incapacity of the crossbred endosperm to maintain its function as a nurse tissue in association with an embryo whose absorptive power is not correspondingly impaired by the hybrid condition. This endosperm-embryo imbalance doubtless hastens death of the 2n×*L. peruvianum* seed. It is not, however, the primary factor in causing it. This is shown by the fact that the hyperplastic condition of the endothelium which, in association with a weak endosperm, regularly leads to collapse has already become established in the seed before any evidence of cellular breakdown in the vicinity of the embryo appears. The endosperm is destroyed the more quickly because there is added to the handicap imposed upon it by a proliferating endothelium the demands of a relatively aggressive embryo. Being inherently incapable of attaining its normally dominant physiological position in the young seed, the hybrid endosperm becomes prey both to the maternal and embryonic tissues which impinge upon it.

A principal question to which an answer is sought in the present investigation—namely, whether the seed failure associated with matings between a diploid and its autotetraploid is comparable in kind to that occurring in an interspecific cross—is answerable in the affirmative. The histological evidence clearly shows that substitution of male gametes from either the autotetraploid (n=24) or *L. peruvianum* (n=12) for normal male gametes in fertilization of an ovule of diploid *L. pimpinellifolium* (n=12) precipitates a common chain of abnormal development events culminating in seed collapse. The seeds resulting from the 4n×2n mating show the same series of histological changes preceding breakdown as 2n×4n seeds. The number of chromosomes in the 4n×2n endosperm is 60 (fig. 20), whereas that in the 2n×4n endosperm would be 48.

In its genetic organization the angiosperm seed is a mosaic. Normally the cells in the maternal tissue and the embryo possess the same number of chromosomes although not necessarily the same genes. The endosperm, whose mother cell is a product of triple fusion, carries an additional chromosome set of maternal origin. In the simplest case, therefore, the endosperm is 3n, whereas embryo and the maternal tissues of the seed are 2n. The chromosome numbers in the three parts of the various classes of tomato seeds under investigation are given below. The symbols "n" and "n_1" refer to the haploid sets of *L. pimpinellifolium* and *L. peruvianum* chromosomes, respectively. The matings which fail to yield plump seeds are boxed.

The evidence is clear in the writers' judgment that the embryo, as an agent

contributing to the present type of seed failure, can usually be ignored. At most its rôle can be only secondary to that of the larger, more active endosperm. It is the latter tissue in its interrelationships with the maternal portion of the young seed that determines primarily whether development succeeds or fails. Persistence of the antipodals as in the juvenile caryopsis of the Gramineae complicates the relationship of the endosperm to the outer tissues of the seed but does not alter its basic significance. A primary requirement for development of the embryo is that the latter establish an efficient connection with a food supply which is mainly outside the seed. In the Gramineae, as in other families, it is the endosperm which mediates the nutritive arrangements (COOPER and BRINK 1944; BRINK and COOPER 1944).

NUMBER AND ORIGIN OF CHROMOSOMES

	(1)		(2)		(3)	
	2n×2n		2n×2n$_1$ (*peruv.*)		2n×4n	
Maternal tissue	24	nn	24	nn	24	nn
Endosperm	36	nn·n	36	nn·n$_1$	48	nn·nn
Embryo	24	n·n	24	n·n$_1$	36	n·nn

	(4)		(5)		(6)	
	4n×4n		4n×2n$_1$ (*peruv.*)		4n×2n	
Maternal tissue	48	nnnn	48	nnnn	48	nnnn
Endosperm	72	nnnn·nn	60	nnnn·n$_1$	60	nnnn·n
Embryo	48	nn·nn	36	nn·n$_1$	36	nn·n

The table above shows that the difference between the viable 2n×2n and the inviable 2n×4n classes of seeds (columns 1 and 3) lies in the latter possessing the tetraploid rather than the triploid number of chromosomes in the endosperm. The chromosome number of the associated maternal tissue in these two cases, of course, is the same. Similarly, the 4n×2n seed (column 6) with the pentaploid number of chromosomes in the endosperm fails, whereas the 4n×4n seed (column 4), differing in having a hexaploid endosperm, succeeds. The ratio of chromosome numbers between endosperm and maternal tissue in the viable 2n×2n and 4n×4n seeds is identical, namely 3:2. Increasing this ratio to 4:2 in the one case and decreasing it to 5:4 in the other, results in failure of the seeds.

There is at present no clue to the reason why these alterations in chromosome balance between endosperm and maternal tissue should radically disturb seed development. Data from numerous sources agree in showing that doubling the chromosome number increases nuclear size, and the change is often very close to proportional. Concomitant enlargement of the cell is usual. It would be difficult to determine in the young endosperm the effect on nuclear and cell size of variations in chromosome number because of the meristematic condition of the tissue and the hypertrophy that commonly occurs, not to mention

the variation in shape of these structures. So we have not attempted it. Casual observation, however, reveals that there is a general tendency in both endosperm and associated maternal tissue for cell and nuclear size to rise as the chromosome number increases. A basis for possible difference in physiological behavior lies in the fact that as nuclear and cell volume are altered, the surface area of these structures does not change proportionately. Were the bodies spherical, the surface area would vary as the two-thirds power of the volume. Assuming that surface-volume relations of nucleus and cell are important in certain metabolic processes, the relatively large differential occasioned by a doubling of volume might well affect growth significantly. A balanced change in chromosome number, as in the tetraploid, does not affect seed development adversely. The incompatibility arises when the chromosome ratio between maternal tissue and endosperm is varied in either direction. If surface-volume relations in cell and nucleus are involved in seed abortion, evidently it is the differential set up across the boundary between endosperm and endothelium which is important.

The mating $2n \times 2n_1$ (*L. peruvianum*) is an example of a cross which leads to seed collapse, although no alteration from the normal in chromosome balance between endosperm and maternal tissue is involved. The literature affords several other instances of the same kind. For convenience the cause of failure in these cases may be termed genic in contrast with the chromosomal type just considered.

Comparison of the $4n \times 2n$ mating in *L. pimpinellifolium* with the $4n \times 2n_1$ (*L. peruvianum*) mating shows that the genic and chromosomal factors affecting seed development may interact to give effects which are not predictable from a knowledge of the action of each factor alone. Seeds from the former mating collapse, whereas many from the latter develop. The essential difference between the two kinds of seeds resides in the genes carried in one of the five sets of chromosomes in the respective pentaploid endosperms (columns 5 and 6). The fact that $4n \times 2n$ seeds abort indicates that a 5:4 ratio in chromosome number between endosperm and maternal tissue is unfavorable. Likewise, collapse of the seeds resulting from the $2n \times 2n_1$ (*L. peruvianum*) cross shows that substitution of a *peruvianum* for a *pimpinellifolium* genome in the triploid endosperm associated with diploid maternal tissue is fatal to seed development. One might expect from these facts that the $4n \times 2n_1$ (*L. peruvianum*) mating would lead to abortive seeds, since it involves a seemingly unfavorable chromosomal balance and an unfavorable genic substitution as well. Such, however, is not the case. Many $4n \times 2n_1$ (*L. peruvianum*) seeds grow to maturity.

It would seem that continued development of the young seed requires the maintenance of a delicate physiological balance between endosperm and adjacent maternal tissue. This balance appears to be upset in one way by altering the chromosomal relations and in a different way by genome substitution. The ultimate effects of both kinds of primary disturbance, however, are alike, since both precipitate the same series of histological changes leading eventually to

seed failure. It must be supposed that the two types of unbalance are opposite in direction to some extent so that when combined, as in the 4n×2n$_1$ (*L. peruvianum*) seed, they tend to cancel each other. These considerations illustrate the difficulty involved in assigning the cause of seed failure when the latter arises in crosses between species which differ both in chromosome number and genic complement.

SUMMARY

A comparative histological study of seed development following reciprocal matings between diploid and tetraploid strains of *Lycopersicon pimpinellifolium* and crosses of these races with *L. peruvianum* is reported.

A high percentage of the flowers pollinated formed mature fruits in all matings except 2n×4n. The flowers and young fruits in the latter case were shed within eight days. The initial set was high in the 4n×4n mating, but numerous immature fruits were shed at intervals up to 24 days.

Practically all the seeds formed following the 2n×2n mating were plump at maturity. About two-thirds of those found in the 4n×4n fruits were sound and the remainder shrivelled. The 4n×*L. peruvianum* cross likewise gave a relatively high proportion of plump, although small, seeds. The seeds in the mature fruits resulting from the 4n×2n and 2n×*L. peruvianum* matings, on the other hand, were much shrunken and incapable of germination.

Double fertilization takes place and both endosperm and embryo start growth in all developing seeds. Fertilization was found to occur approximately 24 hours following pollination of 2n flowers but is somewhat delayed in the 4n flowers regardless of the type of mating.

The endosperm develops rapidly in the 2n×2n and 4n×4n seeds and differentiates early, the peripheral layer of cells acting as an absorbing tissue. The endothelium—that is, the inner layer of cells of the expanding integument adjacent to the endosperm—remains a single layer of densely cytoplasmic cells in the growing seed. Several layers of cells of the integument immediately outside the endothelium become empty of their contents during the early stages of development of the endosperm, thus serving as a source of nutrients.

The chalazal aperture opposite the end of the vascular bundle in conjunction with the endothelium serves as a pathway for nutrients at later stages of development of the endosperm.

The embryo grows at the expense of the endosperm.

The development of the 4n×*L. peruvianum* seed is essentially similar although much slower than that of the 4n×4n seed.

Growth of the endosperm in the 2n×4n, 2n×*L. peruvianum*, and 4n×2n seeds is slow, and the cells at the chalazal end are large and highly vacuolate at 144 hours. By this time there is a conspicuous thickening of the endothelium mainly on the dorsal side of the endosperm. Shortly thereafter the endothelium becomes actively meristematic and grows rapidly so that this tissue has within a few days completely filled the space formerly occupied by the endosperm.

A large amount of deeply-staining material accumulates in the cells of the chalazal pocket during the course of collapse of the aborting endosperms.

The embryo grows slowly and is very small and starved in appearance by the time it has become completely surrounded by the hyperplastic endothelium. Shortly thereafter the embryo dies, and a much-shrivelled seed is found in the mature fruit.

The course of seed failure associated with matings between the diploid and its autotetraploid is very similar to that found following the interspecific cross.

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SUMMARIES FROM FORTHCOMING PAPERS

IRWIN, M. R., and L. J. COLE. *Immunogenetic studies of cellular antigens: individual differences between species hybrids.*—Received March 20, 1945.—Individual differences in antigenic content were observed among the cells of species hybrids of the cross of Senegal × Ring dove. It was found that some of these differences could be explained by the segregation of at least a part of three separate antigenic characters (d-1, d-2, and d-4) peculiar to Pearlneck—as compared with Ring dove—which are also shared by Pearlneck, in whole or in part, with Senegal. Differences in reactivity of reagents prepared from Pearlneck and Senegal antiserums, respectively, showed that a part of the d-2 substance of Pearlneck is shared by Pearlneck and Senegal, but a part is particular to Pearlneck.

GERSTEL, D. U. *Inheritance in Nicotiana tabacum. XIX. Identification of the tabacum chromosome replaced by one from N. glutinosa in mosaic resistant Holmes Samsoun tobacco.*—Received April 2, 1945.—It had been shown previously that mosaic resistance was introduced into Holmes Samsoun tobacco from *N. glutinosa* by substituting a pair of *glutinosa* chromosomes for one of *N. Tabacum.* By morphological and cytogenetical analysis, using the monosomic series of *N. Tabacum*, the replaced chromosomes was identified as the H chromosome.

COOPER, KENNETH W. *Normal segregation without chiasmata in female Drosophila melanogaster.*—Received May 1, 1945.—Approximately 76 percent of the bivalents formed by *Indl-49*, $InB^{M_1}/Dp11_2$ X-chromosome heterozygotes are not conjoined by chiasmata.

In spite of the high frequency of non-exchange tetrads, or bivalents not conjoined by chiasmata, segregation of the X chromosomes is essentially normal in the female.

The chiasma hypothesis of metaphase pairing is not universally applicable, even if the domain generalized by it is specified in such a manner that aberrant forms of meiosis are deliberately excluded.

Mechanisms, other than chiasmata, which may serve to guarantee meiotic conjunction and segregation are briefly discussed.

Recent experimental work interpreted on the basis of the reciprocal chiasmata hypothesis of X-Y conjunction at meiosis in Drosophila males is shown neither to require nor to support that hypothesis.

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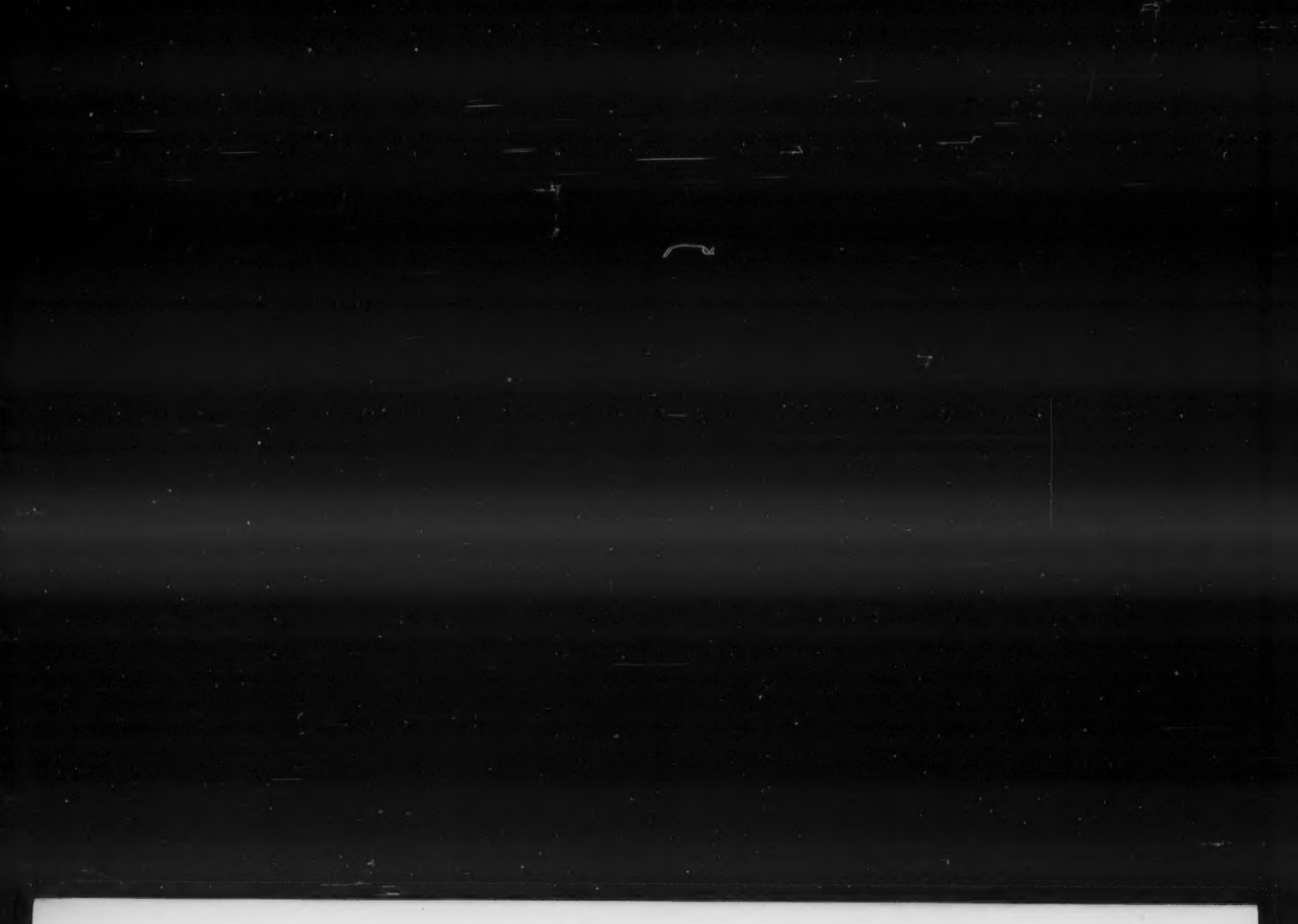
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